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VEGETATIVE PROPAGATION OF DERRIS AND LONCHOCARPUS
WITH THE AID OF GROWTH SUBSTANCESWILLIAM C. COOPER¹

Introduction

The effectiveness of growth substances in inducing root formation on cuttings of the rotenone-bearing plants, *Derris elliptica* var. Sarawak Creeping and *Lonchocarpus utilis*, has been determined to provide information that may be of value in the culture of these crops in the tropics of Latin America. Both species are propagated exclusively by cuttings, so that any treatment which stimulates rooting may be important in their commercial production.

The main emphasis of the present investigation was placed on *Derris*, because of the urgency to develop some propagating procedure that would utilize the large quantities of small stems which have not proved satisfactory in field plantings and are largely discarded as useless. Considerable data, however, have also been obtained on *Lonchocarpus*.

The facilities of the Puerto Rico Experiment Station, U.S.D.A., at Mayaguez, P.R., were made available to the Office of Foreign Agricultural Relations for these investigations. The extensive collections of *Derris* from the Far East and of *Lonchocarpus* from the Western Hemisphere which have been assembled at this experiment station for observation and test made this location ideal for the present investigation. Furthermore, the work was done at a time when this station was actively engaged in distributing large quantities of planting stock of

Derris to growers and experiment stations in Central and South America.

METHODS.—Four synthetic growth substances known to induce root formation on other species of plants were applied to the cuttings by the concentrated-solution dip technique (1). The compounds used were indoleacetic acid, indolebutyric acid, α -naphthaleneacetic acid, and α -naphthalene acetamide. Briefly, the method consists of dipping $\frac{1}{2}$ inch of the base of the cutting for 5 seconds in a 50% ethyl-alcohol solution containing from 1 to 5 mg. per ml. of growth substance. The method was found to be equally as effective in the rooting of *Derris* cuttings as the other standard methods which have been used.

The propagating facilities consisted of muslin-shaded greenhouse benches, sash-covered greenhouse benches, and open-field nursery beds. The greenhouse benches, constructed of wood, were 30 feet long, 3 feet wide, and 1 $\frac{1}{2}$ feet high. They contained approximately 10 inches of a fine yellow sand with a pH of 7.0. The open-field nursery beds were the same as those used by MOORE (4) at the Puerto Rico Station for the propagation of *Derris*. They consist of flat-topped ridges about 8 inches high and 3 feet wide, separated by drainage ditches about 1 foot wide. In these beds the small stem cuttings were planted about 1 inch apart in cross rows 6 inches apart. They were placed apical end uppermost at a 45° angle and almost completely covered with soil.

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Experiments on Derris

Derris, a creeping leguminous plant, sends out numerous vines many feet in length. By the time the roots, which contain the rotenone, are ready for harvest (usually after $1\frac{1}{2}$ –2 years), the vines on top of the ground have grown into a tangled mat. In the case of the Sarawak Creeping variety, which is the principal one planted at Mayaguez, most of the vines on the under side of the mat have shed their leaves and are more or less mature woody stems of varying size. The leafy vines on the top layer of the mat are mostly thin, semihard, green-wood stems.

This mat of leafy and leafless vines is cleared from the field just before harvesting the roots. In commercial practice no attempt has been made to use the green-wood leafy cuttings as propagating material. Only the mature leafless stems are salvaged, and, of these only the larger stems the diameter of a pencil ($\frac{1}{4}$ inch) have proved satisfactory planting material for open-field nursery beds. Usually only about 10–30% of the thin stems produce plants, while 90% or more of the larger stems are productive. Many of the thin-stemmed cuttings rotted in the beds. Others formed shoots at the top but few if any roots, and eventually died. It was thought that a growth-substance treatment might stimulate the rooting of these leafless small-stem cuttings and thereby produce a higher percentage of good plants than is obtained without treatment.

LEAFLESS CUTTINGS

EFFECTIVENESS OF GROWTH SUBSTANCES ON ROOTING.—The cuttings were prepared in the usual manner as practiced at the Station (4). This consists in cutting the vines into 3-foot lengths, arranging them into bundles of 300 each

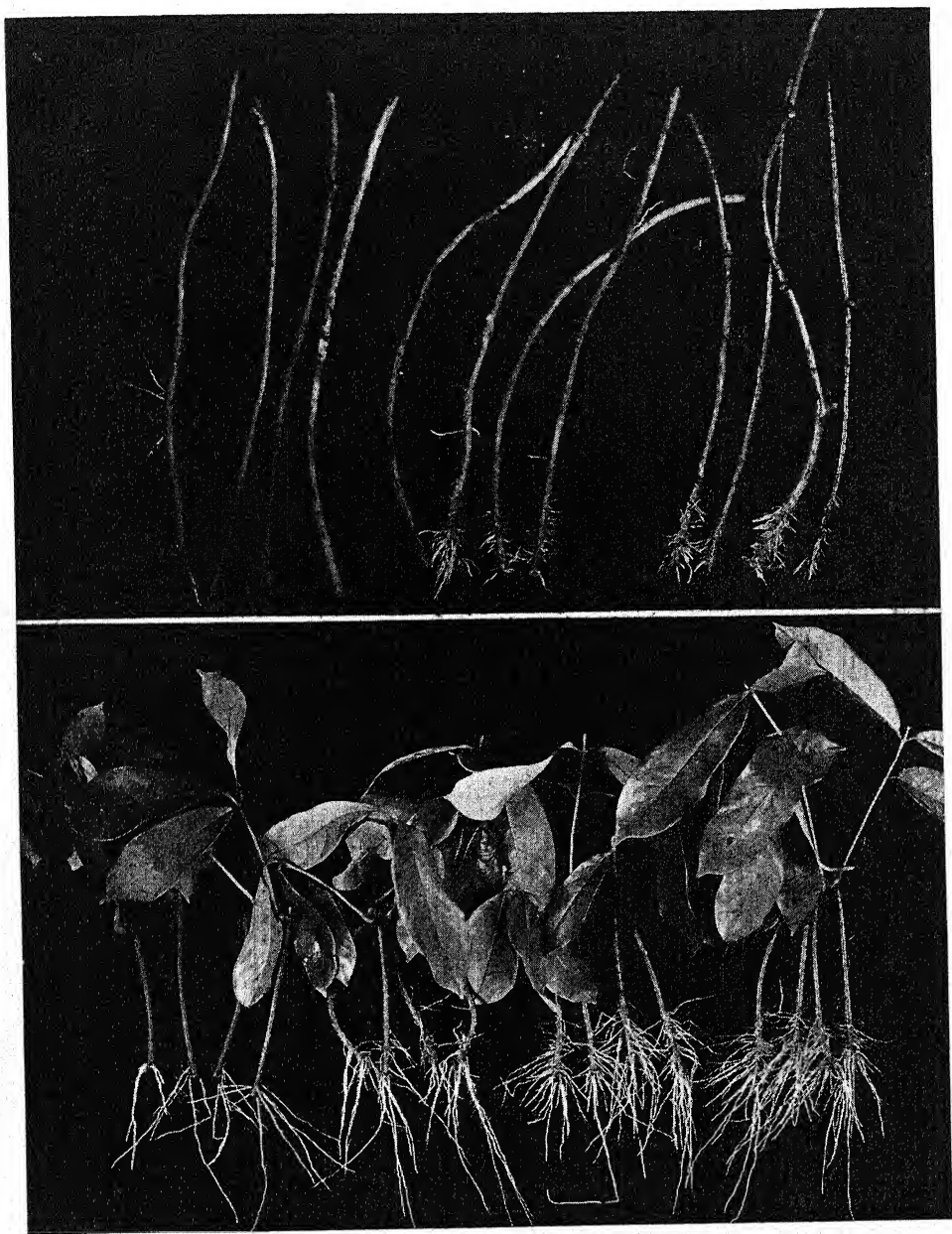
with basal ends together, tying the bundles securely at 6-inch intervals, and sawing them into bundles of cuttings 12 inches long.

A bundle of 300 cuttings was used for each treatment. Each growth substance was tested at 0, 1, and 2 mg. per ml. of solution. The cuttings were planted at once in field nursery beds, and twenty-five were dug up from each lot at 1-week intervals for examination.

At the end of 2 weeks the controls had formed an average of 2.4 roots per cutting on 33% of the cuttings, the remaining 67% having no roots. Those indole-acetic-acid treated showed no increase in rooting over the controls, while cuttings treated with indolebutyric acid, naphthaleneacetic acid, and naphthalene acetamide showed considerable increase in rooting. All three substances induced roots on approximately 75% of the cuttings when used at a concentration of 1 mg./ml. and on 88–100% when used at 2 mg./ml. The number of roots induced varied considerably but averaged about twenty for the 1 mg./ml. and thirty for the 2 mg./ml. solutions.

There appeared to be no very significant difference in the effectiveness of the three substances at these concentrations. Naphthaleneacetic acid at the 2 mg./ml. concentration did cause slight injury to the base of the cuttings, however, while no injury was noticeable at this concentration for the other two substances. Typical cuttings treated with the indolebutyric-acid series are illustrated in figure 1.

The cuttings used in the preceding experiments were selected for uniformity and were classed as mature stems with yellow, or partially etiolated, bark. In any given bundle of field-run cuttings there may be other types of stems that respond differently to treatment. In par-



FIGS. 1, 2.—Fig. 1 (above), leafless cuttings of *Derris* 3 weeks after treatment with indolebutyric acid. Left to right: 50% alcohol (control); 1 mg./ml.; and 2 mg./ml. Planted in silty-clay loam. Fig. 2 (below), comparative growth of root systems on leafy and leafless cuttings 5 months after treatment with indolebutyric acid. Left to right: leafy 50% alcohol (control); leafy 2 mg./ml.; leafy 5 mg./ml.; leafless 50% alcohol (control); and leafless 2 mg./ml. Tops removed before photographing.

ticular, green stems were found to require higher concentrations to induce rooting than was required for the yellow-bark type of stem. The minimum effective concentration of indolebutyric acid for green stems was found to be 2 mg./ml.

AFTEREFFECTS OF GROWTH SUBSTANCES.—Data obtained from a detailed inspection of fifty or more of

saw cut, and planting was in a silty, well-drained loam. Under these conditions both control and indolebutyric acid (2 mg./ml.) treated cuttings grew better than in the regular field experiments, but the percentage of good plants after 3 months was less for the treated plants (49%) than for the controls (71%). As illustrated by the three cuttings on the right in figure 5, there was

TABLE 1

EFFECT OF GROWTH SUBSTANCES ON SURVIVAL OF LEAFLESS SMALL-STEM CUTTINGS OF DERRIS PLANTED IN THE FIELD

Exp. No.	GROWTH SUBSTANCE	CONCENTRATION (MG./ML.)	MONTHS AFTER PLANTING	No. OF CUTTINGS INSPECTED	PERCENTAGE WELL-ROOTED WITH LEAFY TOP		PERCENTAGE DEAD	
					Control	Treated	Control	Treated
1.	Naphthaleneacetic acid.....	1	4	50	27	39	33	33
2.	Naphthaleneacetic acid.....	2	4	50	27	45	33	18
3.	Naphthaleneacetic acid.....	1	4.5	50	32	40	16	23
4.	Naphthaleneacetic acid.....	2	4.5	50	32	29	16	15
5.	Naphthaleneacetic acid.....	2	4.5	50	32	10	16	65
6.	Naphthalene acetamide.....	2	3	80	69	31	24	51
7.	Indolebutyric acid.....	1	4	79	24	20	35	39
8.	Naphthaleneacetic acid.....	1	4	82	24	32	35	33
9.	Naphthaleneacetic acid.....	2	4	91	24	32	35	43
10.	Naphthaleneacetic acid.....	2	4	56	24	5	35	55
11.	Naphthalene acetamide.....	1	4	67	24	33	35	48
12.	Naphthalene acetamide.....	2	4	85	24	12	35	49
Average.....					30	29	32	40

treated and control plants 3-4½ months old from twelve different locations on the experiment station are given in table 1. These data show that the average percentage of well-rooted plants with vigorous top growth was low, only 30%, and was the same for treated and controls. The actual number of cuttings that had died was greater for the treated (40%) than for the control lots (32%).

These results obtained in the field were checked under slightly more favorable conditions, whereby the cuttings were carefully graded, a smooth knife-cut at the base was substituted for the

no sign of stem injury from the treatment. Most of the roots induced by the treatment simply stopped growing after they reached about ½-1 inch in length.

LEAFY CUTTINGS

EFFECTIVENESS OF INDOLEBUTYRIC ACID ON ROOTING.—The leafy cuttings used were similar to the greenwood leafless cuttings, except that one leaf, trimmed to three leaflets, was left at the apex of each cutting. Only indolebutyric acid was used. After treatment, the cuttings were inserted in sand in the sash-covered greenhouse benches, since pre-

vious trials had shown that the leaves could be kept alive only in closed sash-covered frames.

Figure 2 shows that control leafy cuttings produced about five roots per cutting after a 3-week period in the frames. Treatments with 1, 2, and 5 mg./ml. concentrations of the acid were definitely effective in increasing the number of roots as compared with the controls. From root counts made on twenty-five cuttings of each treatment, the average number of roots per cutting for 1 mg./ml. was twenty-five; for 2 mg./ml. was thirty-four; and for 5 mg./ml. was sixty. No injury at the base of the cuttings was induced by any of these treatments.

The 2 mg./ml. solution was also used on leafless as well as on leafy cuttings, and this solution induced only about half as many roots on leafless stems as on leafy ones (fig. 3). Both types of cuttings were grown in the sash-covered frame.

AFTEREFFECTS OF GROWTH SUBSTANCES.—Rooted leafy cuttings grew well when transplanted directly from the propagating frames to the field nurseries. Figure 4 shows the controls and the treated lot (5 mg./ml.) 1 month after transplanting. All roots on both lots were growing vigorously. There was no sign of root degeneration.

Figure 5 shows a comparison of root growth on treated and control leafy and leafless cuttings 5 months after planting. The roots on the leafy cuttings were still growing vigorously. These plants were from the same lot of cuttings as illustrated in figures 2 and 4. The root system on the treated cuttings was much more extensive than on the controls.

In comparing the treated leafy and leafless cuttings (top growth removed from all plants to facilitate photographing), it is seen that while root growth was vigorous and extensive on treated

leafy cuttings, it was very weak on treated leafless ones. Most of the roots induced by the treatment on leafless cuttings had either died or were in about the same state of growth as they were 4 months earlier. Thus it appears that vigorous root growth on treated cuttings was associated with the presence of leaves.

Another beneficial after-effect resulting from the treatment of leafy cuttings is the greater survival of the plants, as compared with controls, on transplanting. In a large-scale experiment, 2000 untreated leafy cuttings and 2000 treated (5 mg./ml.) cuttings were transplanted to field nursery beds after a 3-week period in the propagating frames. The transplanting took place during a dry hot spell in late August, and it was necessary to apply partial shade for 3 days. Counts made on leaf-drop 10 days after transplanting showed that 95% of the treated plants held their leaves and were growing well as compared with 85% for the untreated cuttings. The fifty-five more roots per cutting on the treated plants (five roots on the controls) appeared to be a definite aid in quick establishment in the new environment.

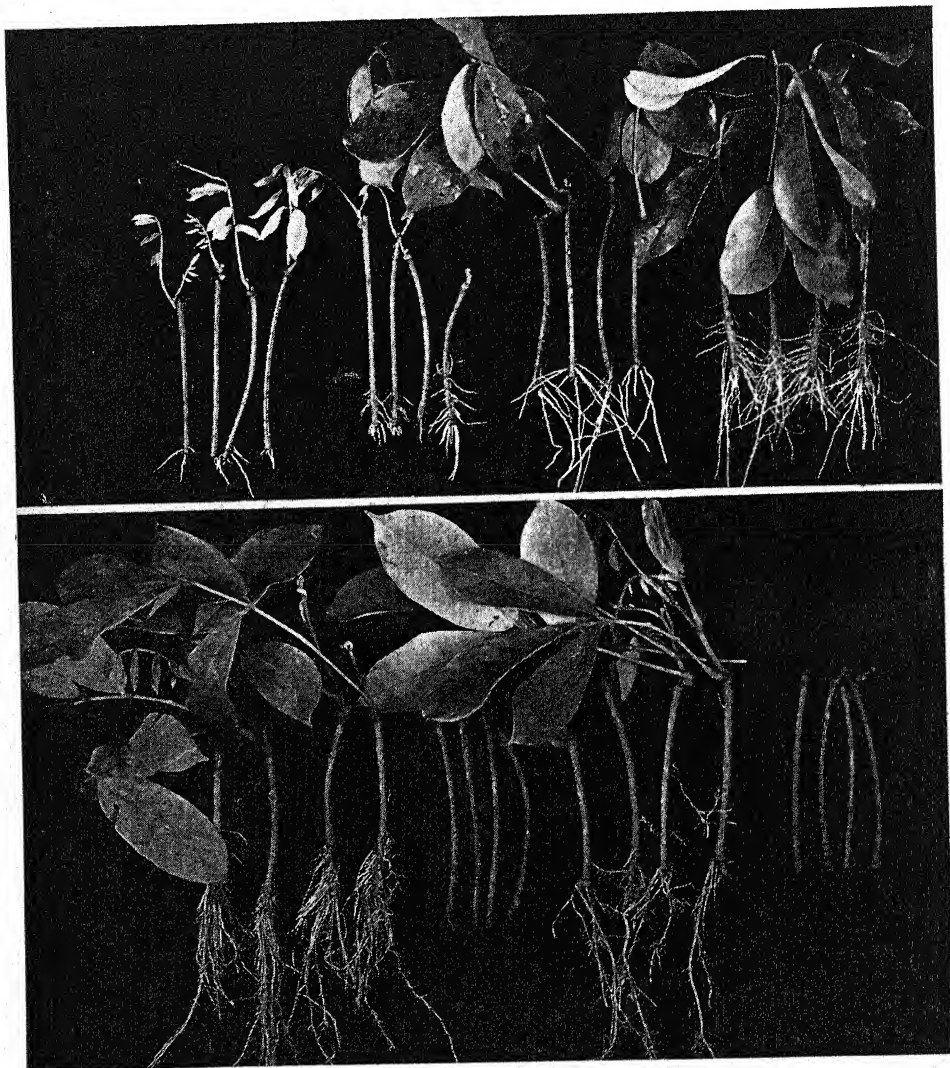
Experiments on *Lonchocarpus*

Lonchocarpus, like *Derris*, is a member of the legume family. It has an erect growth habit, becoming almost treelike after 2 years (fig. 6). The plant, native to South America, furnished about 50% of the United States supply of rotenone before the war and is today our principal source of this material. The two high rotenone-yielding clones of *Lonchocarpus utilis* growing at the Puerto Rico Station, and with which this investigation was concerned, are reported (3) to have rotenone contents of 14.02 and 16.5%.

The plant is propagated by stem cut-

tings made by sawing the long woody stems, usually 1 inch or more in diameter, into cuttings that have several

In South America it is the usual practice to set the cuttings directly in the field, where mortality up to 50% is not



FIGS. 3, 4.—Fig. 3 (above), leafy cuttings of *Derris* 3 weeks after treatment with indolebutyric acid. Left to right: 50% alcohol (control); 1 mg./ml.; 2 mg./ml.; and 5 mg./ml. Fig. 4 (below), comparative rooting of leafy and leafless cuttings of *Derris* 3 weeks after treatment with 2 mg./ml. indolebutyric acid. Left to right: leafless control, leafless treated, leafy control, leafy treated.

nodes and are 12–18 inches in length (5). In this investigation, however, owing to shortage of propagating material, the stems were sawed into 6-inch lengths.

unusual (5). In Puerto Rico the mortality of field-planted cuttings of the high-yielding clones was much greater than 50%, and often none of them survived.

Accordingly, the greenhouse benches were used exclusively in this investigation.

LEAFLESS STEM CUTTINGS

The four growth substances used in the experiments with *Derris* were tested on leafless cuttings of *Lonchocarpus* at 0, 1, 2, and 5 mg./ml. concentrations.

bases. The results were complicated, however, in that a great many of the cuttings succumbed from a decay (undetermined species of *Penicillium*) occurring at the top of the cuttings. The first evidence of this mold at the apex occurred within 6 days after placing the cuttings in the propagator. It was often preceded by a resinous exudate from the

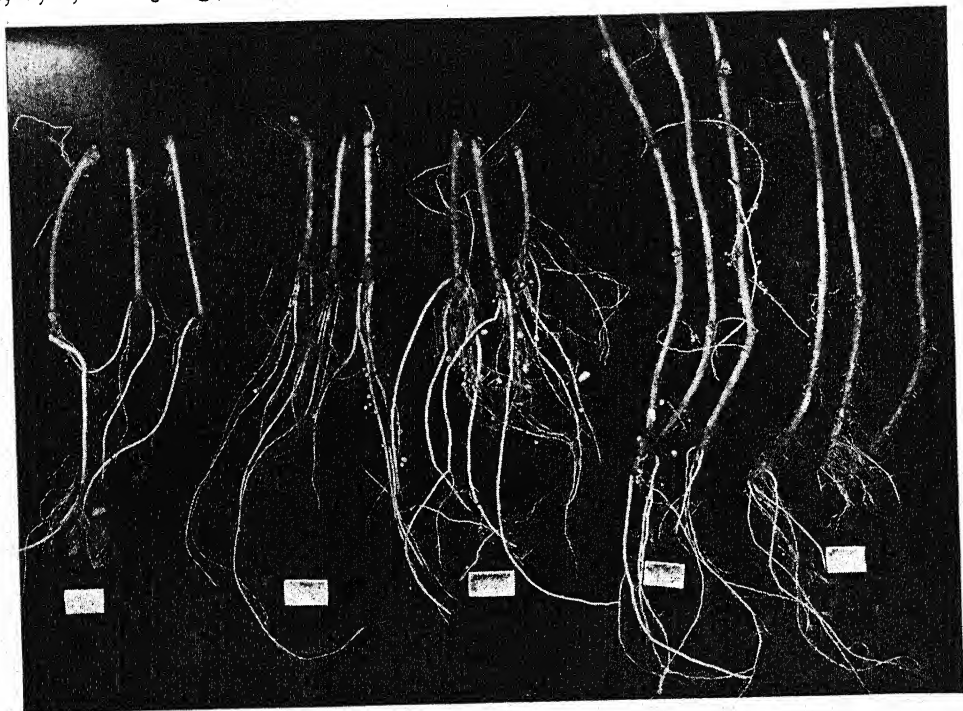


FIG. 5.—Leafy cuttings of *Derris* 1 month after transplanting to nursery beds. Plants on left: 5 mg./ml. indolebutyric acid; plants on right: 50% alcohol (control).

The control cuttings developed an average of about five roots each. The indoleacetic acid at all concentrations failed to increase the number of roots over that in the controls. On the other hand, the three other compounds were especially effective in stimulating rooting. The 1 mg./ml. solutions showed a slight increase in the number of roots, while the 5 mg./ml. solution of all three substances induced as many as fifty husky roots per cutting without causing noticeable injury to the

trachea tubes at the upper cut surface. The bark around the top of the cuttings was usually killed within 2 or 3 weeks.

In general, the decay at the top occurred more frequently on treated cuttings than on controls. It was definitely greater on cuttings treated with a 5 mg./ml. solution of indolebutyric acid (table 2; fig. 7). At the same time there was pronounced bud inhibition in the treated cuttings. It may be that the decay is in some way connected with the

bud inhibition induced by the treatment.

Cutting material was so limited that it was not possible in this investigation to determine whether a growth-substance treatment could be developed that was effective in increasing root formation

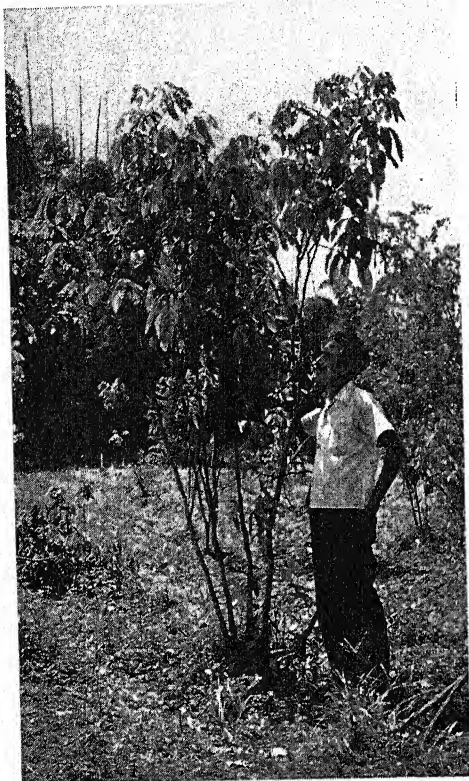


FIG. 6.—Typical plant (3 years old) of one of the high rotenone-yielding clones of *Lonchocarpus* now growing at Puerto Rico Experiment Station.

without inducing excessive bud inhibition and top decay. Until this problem is further investigated it seems inadvisable to use the growth substances on this type of cutting. Untreated material, under the conditions of these experiments, usually developed one or more (average five) roots on approximately 70% of the cuttings after 6–8 weeks and at the same time produced vigorous top growth.

LEAFY VINE CUTTINGS

Plants of *Lonchocarpus* after 2–3 years of upright growth will frequently

TABLE 2

NEW SHOOT GROWTH, NUMBER OF ROOTS, AND INCIDENCE OF DECAY IN NAPHTHALENE-ACETAMIDE TREATED AND CONTROL CUTTINGS OF *LONGHOCARPUS*

Concentration (mg./ml.)	No. of weeks buds dormant	Ave. length of new shoots after 7 weeks (cm.)	Ave. no. of roots per cutting after 7 weeks	Percentage cuttings showing top decay
0.....	2	68.0	4.9	10
5.....	6	c. 5	50.0	60

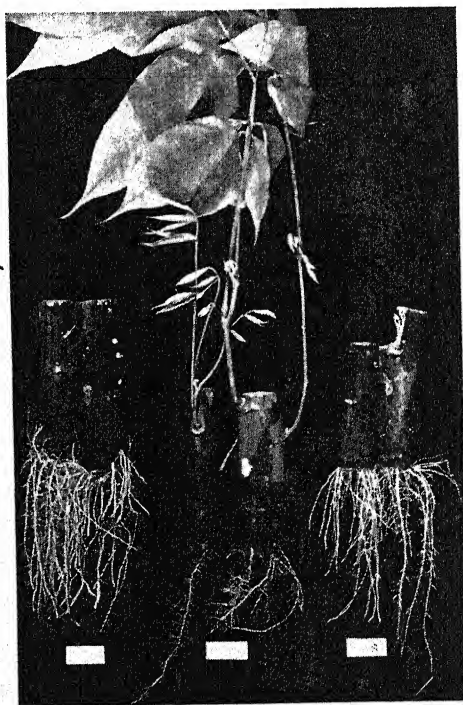
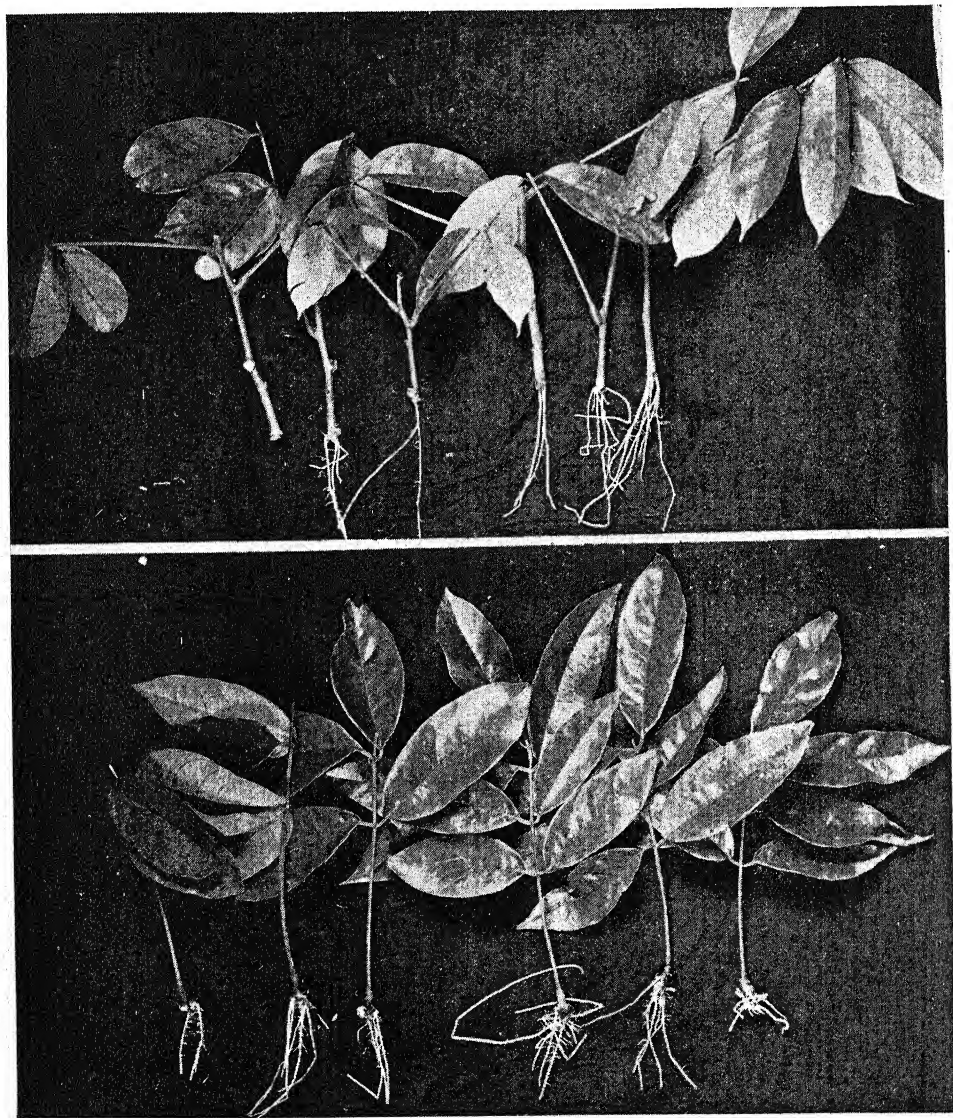


FIG. 7.—Leafless cuttings of *Lonchocarpus* 6 weeks after treatment with α -naphthalene acetamide. Left to right: 5 mg./ml.; 50% alcohol (control); 2 mg./ml.

produce extensive vinelike growth at the top of the plant. Such vines can be cut into a number of pieces containing one

or more nodes with a leaf at the apex and be used as cuttings. Rooting obtained on treated and untreated leafy

butyric acid induced an average of five roots per cutting as compared with two for untreated cuttings.



FIGS. 8, 9.—Fig. 8 (above), leafy vine cutting of *Lonchocarpus* 3 weeks after treatment. Left to right: 50% alcohol (control); 2 mg./ml. of indolebutyric. Fig. 9 (below), leaf-bud cuttings of *Lonchocarpus* 3 weeks after treatment. Left to right: 50% alcohol (control); 2 mg./ml. indolebutyric.

vine cuttings after 3 weeks in sash-covered frames is shown in figure 8. A treatment consisting of 2 mg./ml. indole-

It has not yet been determined into what type of plant these leafy vine cuttings will develop, and an opinion as to

their value as propagating material is reserved until further investigation.

LEAF-BUD CUTTINGS

Another method of vegetative propagation now being used extensively, particularly in ornamental plants, is leaf-bud cuttings (6). The technique consists of taking a bud with a single leaf attached, just as though it were to be used in shield budding. The bud is inserted in sand so that the shield of wood is just covered and the leaf is exposed above the sand. In these experiments the leaf-bud cuttings were placed in the sash-covered frames.

Results obtained with treated and untreated leaf-bud cuttings are illustrated in figure 9. The leaf buds were taken from the leafy shoots at the top of the plants and not from leafy vines. The cuttings were treated by dipping the bud and the lower part of the petiole in the growth-substance solution, which in this experiment consisted of indolebutyric acid, 2 mg./ml. In 4 weeks the leaf buds produced about five long heavy roots, and there was no great difference in the number produced on treated and control cuttings. The buds on these cuttings had not started to grow after 1 month but were still alive and healthy. If these buds develop satisfactorily, this method has a definite application where there is scarcity of propagating material. Each node of the leafy top growth of the plant (fig. 6) may be used as a leaf-bud cutting.

Discussion

Under the conditions employed in these experiments, the hormone treatment of leafless cuttings of *Derris* and *Lonchocarpus* appears to be more detrimental than beneficial. All three substances were effective in inducing roots,

but the cuttings failed to develop properly.

In case of *Derris*, most of the roots simply failed to grow beyond an inch or so in length. Often the roots would wither and die. Always accompanying this rooting response was pronounced bud inhibition. In *Lonchocarpus* there was the same abundance of roots induced and the same pronounced bud inhibition. But there was an additional complicating factor resulting from invasion of the cuttings at the apex by a *Penicillium* mold that usually killed the cuttings within 6 weeks.

This collapse of leafless cuttings of *Derris* and *Lonchocarpus* as a result of treatment was induced without any evidence of external injury to the tissues of the base of the cutting. Such killing of the base can be induced by the use of more concentrated solutions. The disturbance concerned with in this discussion is apparently an internal physiological one created by the treatment and apparently associated with excessive numbers of roots.

It has been demonstrated that growth-substance treatment apparently causes mobilization of food reserves at the base of cuttings, where they are utilized in root formation (2). In treated leafless cuttings, where fifty or more roots are frequently initiated on a single cutting, there is bound to be a heavy demand for the stored food materials. With leafy cuttings this demand can be taken care of by continued photosynthesis in the leaves. In leafless cuttings there is no way to obtain additional food supply except by pushing out new shoots. In itself, however, the treatment induces temporary bud inhibition. Thus the collapse of treated leafless cuttings of *Derris* may be due, at least partly, to the starva-

tion caused by the excessive number of roots depleting the reserve food supply.

Decay of the treated cuttings of *Lonchocarpus* was hastened by the entrance of the *Penicillium* at their apex, indicating that somehow the treatment made the cuttings more susceptible to attack; but whether treatment directly stimulated growth of the decay organism is not known.

Recently STUART and MCCLELLAN (7) reported that the severity of narcissus basal rot was increased by the use of growth substances and that the substances stimulated the growth of pure cultures of *Fusarium oxysporum*, the organism causing basal rot. They inferred that the basal rot of the treated bulbs was due to the stimulating effect of the substances on growth of the organism.

From this conclusion it might be suspected that the growth substances stimulated the *Penicillium* decay in *Lonchocarpus*. At the same time the explanation for the increased incidence of decay might lie in the increased susceptibility of the apex of the cutting due to depleted food reserves in that section. Once the buds began to grow there was usually no more decay.

Use of more dilute solutions on leafless cuttings should be considered. Perhaps the ideal growth-substance effect on leafless cuttings might be to produce only a slight increase in number of roots over controls and not to induce sufficient roots to cause ill after-effects. In general, however, the range in concentration between no increase in rooting and excessive rooting is rather small with leafless *Derris* and *Lonchocarpus*. Also, in any given bundle of field-run cuttings there may be several types that respond differently to the treatment. At the present

stage of investigation it would not be advisable to use a treatment stronger than 0.5 mg./ml. for leafless *Derris*. With leafless *Lonchocarpus* perhaps no treatment should be used until the problem has been further investigated.

Summary

1. Cuttings of *Derris* and *Lonchocarpus* were treated with indoleacetic acid, indolebutyric acid, α -naphthaleneacetic acid, and α -naphthalene acetamide by the concentrated-solution dip technique.
2. Indoleacetic acid did not stimulate rooting at concentrations of 1 and 2 mg./ml., while the other three compounds were highly effective at these concentrations on both *Derris* and *Lonchocarpus*.
3. Roots produced on treated leafless cuttings of *Derris* did not grow well, while those induced on treated leafy cuttings grew exceptionally well.
4. The incidence of decay at the top of leafless cuttings of *Lonchocarpus* was greater in treated than in untreated cuttings.
5. Growth substances stimulated the rooting of leafy cuttings made from the vinelike growth at the tops of *Lonchocarpus*.
6. Treated and untreated leaf-bud cuttings of *Lonchocarpus* rooted well.

The writer is indebted to Dr. KENNETH A. BARTLETT and Dr. RUFUS H. MOORE of the Puerto Rico Experiment Station, U.S.D.A., Mayaguez, P.R., for their generous co-operation during the course of these experiments.

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VARYING STRUCTURE OF CONIFER LEAVES IN DIFFERENT HABITATS

ERNEST L. STOVER

Introduction

Studies of variations in the structure of broad-bladed leaves of plants in different habitats have been made by CLEMENTS (4), STARR (16), HANSON (6), HAYDEN (8), McDUGALL (12), and others. These investigations have shown that the leaves of xeromorphic forms of nonsucculent mesophytes are thicker, have more rows of palisade cells, more sclerenchyma, a thicker cuticle, and more mucilaginous and resinous substances in the mesophyll cells than in the mesomorphic forms. The mesomorphic leaves have larger air spaces than the xeromorphic leaves. The xeromorphic broad leaves develop under conditions of bright light, dry air, and low water supply—or some combination of these factors.

A number of investigators have shown that leaves with many palisade cells and small air spaces (xeromorphic forms) transpire more water per unit of epidermal surface than leaves with larger air spaces (1, 2, 3, 7, 11, 14, 17).

TURRELL (17) has measured the internal leaf surface of various plants and

found that the more compact leaves with numerous small air spaces and usually more layers of palisade cells have greater internal evaporating surface than leaves with larger air spaces. Other things being equal, this greater internal surface of xeromorphic leaves can account for their greater transpiring power.

Studies have been made of the differences in the structure of coniferous leaves of nursery stock by KORSTIAN (9) and LARSEN (10). It was found that conifer leaves grown in shade are thinner, have a thinner epidermis and cuticle, more sponge tissue, and the guard cells are nearer the surface of the leaf than in those grown in brighter light.

The writer has been interested in the differences in the structure of conifer leaves as affected by both age and habitat. The summer of 1940 was spent in the University of Wyoming Science Camp in the Medicine Bow Mountains, and there studies were made of the leaves of *Abies lasiocarpa* (Hook.) Nutt., *Picea engelmanni* (Parry) Engelm., and *Pinus contorta* Loud. The leaves of *A. lasio-*

carpa and *P. engelmanni* were collected from both the lee and windward sides of the Krummholz at about 11,000 feet, from a mesophytic protected area between 9000 and 10,000 feet, and from the dry granite outcroppings east of Laramie known as the Veedavoo Rocks. The specimens of *P. contorta* were collected from the most mesic and from the most xeric habitats found in the area. The xeric habitat was in the Veedavoo Rocks in the Laramie Range and the mesic habitat was in the Medicine Bow Mountains between 9000 and 10,000 feet.

In choosing specimens, care was taken to select average-sized leaves from a given tree. Comparisons are of leaves produced during the same year, since the size of needles varies a great deal from year to year.

In most textbooks the pine needle is the only conifer leaf figured, and usually only the cross-section is shown. So far as the writer knows, the last edition of the STRASBURGER text was the first to figure a portion of the longitudinal section of a pine needle. Longitudinal sections show that the "infolded cells"¹ are palisade-like. Cross-sections of a 2- or 3-year-old pine needle show the largest air spaces (stomatal cavities) adjoining the stomatal openings. But the air spaces in pine needles are much more extensive, for the longitudinal sections from both the xeric and mesic habitats show that the infolded cells of both pine and spruce are transverse plates of cells with varying sizes of air spaces between them.

The mesophyll of the alpine fir is totally without the infolded cells. These leaves show palisade-like cells in trans-

verse as well as in longitudinal sections. This fir grows in relatively mesic habitats, and transverse as well as longitudinal leaf sections show numerous and relatively large air spaces.

The specimens of the three species investigated did not have more than two resin ducts. Those of *Picea engelmanni* are only in the basal half of the needles. The epidermal cells are thick-walled in all specimens except those of the current growing season. These young leaves show thickened cuticle, epidermal cell walls, and thickening of the hypodermal cell walls by the time the leaves reach their maximum size. The greatest thickening of cell walls occurs at the tip and at the base of the leaves, and the greatest number of cells of hypodermal sclerenchyma is at the base of the needles. Thickness of the cuticle varies with age and habitat. The thick-walled hypodermal cells vary in number with the species, the age of the needle, and the habitat. The smallest number occurs in the younger needles and in those growing in the mesic habitats. It is not known how new hypodermal cells originate; that is, whether they are from the division of other hypodermal cells or from the division of epidermal cells.

In the leaves of *Pinus* and *Picea* studied the endodermis is a continuous sheath of cells, and no "passage" cells were found. The endodermis in *Abies* is not a complete sheath of like cells, even in the bases of the leaves (fig. 11). As reported previously by SOAR (15), no Casparian strips were seen in either *Abies* or *Picea*. SOAR also reported that the radial walls of the endodermis were lignified and covered with a layer of suberin, and that the outer tangential walls are thicker than the inner in the specimens examined. SOAR found that the outer tangential walls of the endo-

¹ Species of *Pinus*, *Picea*, and *Larix* are the conifers in North America that have the "infolded" mesophyll cells. There are only a few real "infoldings" in *Picea engelmanni*.

dermis in most leaves of *Pinus* and *Picea excelsa* were lignified and the inner walls sometimes lignified, but that the inner walls may be of pure cellulose. The writer did not find that lignification occurred as SOAR has reported, for in these specimens the inner tangential walls and the radial walls are the first to be lignified, and the degree of lignification increases with the age of the leaves in both mesic and xeric habitats.

The pericycle (transfusion tissue) is made up of three types of cells: cells with bordered pits like those of the tracheids; very thick-walled fiber cells more abundant in xeric habitats; and larger parenchyma cells that retain their cell contents and are often filled with an amorphous substance. All these cells become completely lignified in the oldest and more xeromorphic leaves.

The xylem and phloem in these species of *Pinus* and *Abies* are in two areas of the stele and in *Picea* are in one area, although the phloem is separated into two areas by a prominent phloem ray—except at the base of the leaf.

Methods

Measurements of the leaves were made with Vernier calipers. Living leaves direct from the trees were measured before any shrinkage took place as the result of transpiration. Free-hand sections, both cross and longitudinal, were examined on the day the leaves were collected. Material for further study and for making the stained microscopic slides was preserved in alcohol and formaldehyde with Tergitol. Three cubic centimeters of formaldehyde (40%) was added to each 100 cc. of 30% alcohol. One drop of Tergitol penetrant 7 (Carbide and Carbon Chemicals Corporation) was added to each 100 cc. of

the solution. The leaves were held in this for several months.

The leaves were dehydrated by using cello-solve, two changes of 2-3 hours each, and infiltrated with paraffin by means of toluene to which was added a small amount of chloroform (about 1 cc.). The amount of chloroform can be increased to speed up infiltration. The paraffin has a melting point of 56° C. The imbedding mixture, locally called rubber-paraffin, is the 56° paraffin to which was added a mixture of paraffin, raw rubber, and bayberry wax. The imbedding formula is modified from that of HANCE (5). Twenty grams of raw rubber is dissolved in 100 gm. of paraffin and to this is added 2 gm. of bayberry wax. The imbedding paraffin is made by adding 5-10 gm. of the rubber-bayberry mixture to each 100 cc. of melted 56° C. paraffin.

The sections were fixed to the slides with the Szorndathy fixative. Some slides were stained with safranin and fast green, and others with the Haidenhain haematoxylin iron-alum method. Dehydration during staining was by cello-solve, with clearing in wintergreen oil and xylene.

Investigation

ABIES LASIOCARPA (HOOK.) NUTT.

Table 1 shows that the size of leaves of this species varies as do broad leaves, but within smaller limits. The needles in the most mesic habitat are the longest and thinnest and those in the most xeric habitat the shortest and thickest. The most unexpected difference occurs in the needles of the trees of the Krummholz. There the needles of the windward (west) side of these wind-blown trees have more nearly the size of the leaves from the mesic habitat than have those of the lee

(east) side. This unexpected mesomorphic condition is shown also in the internal structure (table 2).

The abscission of leaves was found to vary with the habitat. The leaves remain on the trees for the greatest number of years in the most mesic habitat, up to 17 years. They remain on the trees for the shortest time in the most xeric habitat. These live for as long as 10 years but many fall before that. The oldest needles found on the windward side of the Krummholz were 12 years old, while those on the lee side live as long as 16 years. This seems to contradict the facts

the alpine fir needles from the same habitats as listed in table 1. The thickness of the cuticle is greatest in the most xeric habitat and tends to be thicker on the lee side of the Krummholz than on the windward side. The stomatal pits are shallowest in the mesic habitats, deepest in the most xeric, and have the same average depth on both sides of the Krummholz. Wall thickness of the epidermal and hypodermal cells is most pronounced in the leaves from the xeric habitats. The subsidiary cells of the guard cells show this variation to a marked degree. The outer epidermal

TABLE 1
ABIES LASIOCARPA, SIZE OF LEAVES

HABITAT	ONE YEAR OLD	TWO YEARS OLD	THREE YEARS OLD	FOUR YEARS OLD	AV. SIZE FOR FOUR YEARS
	Length×Width×Thickness (mm.)	Length×Width×Thickness (mm.)	Length×Width×Thickness (mm.)	Length×Width×Thickness (mm.)	Length×Width×Thickness (mm.)
Alpine: Krummholz, exposed or windward side, 11,000 ft.	20.3×1.55×0.76	21.8×1.52×0.73	22.8×1.47×0.72	22.7×1.47×0.77	22×1.5×0.77
Alpine: protected or lee side	19.5×1.45×0.82	18.5×1.45×0.75	20.7×1.6×0.85	21.5×1.57×0.82	20×1.5×0.81
Mesic: near stream, 9000-10,000 ft.	27×1.5×0.65	26.1×1.5×0.7	23.3×1.5×0.65	25×1.4×0.65	27×1.5×0.66
Xeric: dry rocks, Laramie Range, 6000-7000 ft.	17×1.5×0.83	17.1×1.5×0.82	19.5×1.4×0.76	18.7×1.4×0.8	18×1.4×0.8+

of size and internal structure, which indicate that the leaves on the windward side of a Krummholz are more like those from a mesic habitat than those in the lee side. Abscission is apparently controlled by factors not directly concerned with those causing variations in size and internal structure of evergreen needle leaves.

The difference in thickness of the leaf is more evident to the eyes in the xeric habitats than the measurements would indicate. Leaves in the mesic habitats are always much flattened and often have the appearance of a midrib and lamina, while in xeric habitats the lower surface is more nearly a half cylinder.

Table 2 gives the approximate measurements of the internal structures of

cell walls are thicker than the inner in all leaves (figs. 1-3).

The greatest number of palisade-like cells and the smallest air spaces are in the leaves from the xeric habitats, as shown in the longitudinal sections (figs. 4-6). There is a difference in the size of the resin ducts, not only in different leaves but also in different habitats. The ducts of the smallest diameter are in the leaves from the most mesic habitats (fig. 1).

The veins are largest in the basal halves of the leaves; the measurements in table 2 are averages giving the approximate size of the largest cross-sections from each habitat. The largest veins are in the leaves from the xeric habitat. They are smaller in the most

TABLE 2
ABIES LASIOCARPA, INTERNAL STRUCTURE OF LEAVES

HABITAT	AV. THICK- NESS OF CUTICLE (μ)	AV. DEPTH OF STOMA- TAL PIT (μ)	AV. THICK- NESS OF HYPODER- MAL CELL WALLS (μ)	GREEN MESOPHYLL, PALISADE-LIKE CELLS IN CROSS- SECTION	MESOPHYLL IN LONGISECTION	AV. DI- AMETER OF RESIN DUCTS (μ)	SIZE OF VEIN IN CROSS-SEC- TION		AV. NUMBER OF XYLEM CELLS	AV. NUMBER OF PHLOEM CELLS	REMARKS
							Thickness \times Width (μ)	Thickness \times Width			
Alpine: Krumm- holz, windward side, 11,000 ft.	3.6-4.8	24	2.4-3.6	3+ layers, up- per and lower	Larger air spaces than on lee side	40	270 \times 360	4 \times 8(1) $\frac{1}{2}$ 6 \times 12(2) 6 \times 12(3)	6-7 \times 8-9(1) 9-10 \times 12-15(2) (outer crushed cells cannot be counted) (3)	Phloem cells with thick- ened walls	
Alpine: Krumm- holz, lee side, 11,000 ft.	3.6-7.6	24	1.2-3.6	2-3 layers, up- per and lower	More compact than on wind- ward side	30	250 \times 420	6-7 \times 12-13(1) 5 \times 11-12(2) 6-7 \times 11-12(3)	9-10 \times 11-12(1) 6-7 \times 13-15(2) 9-10 \times 13-15(3) (some crushed and de- stroyed)	Phloem cells with thick- ened walls	
Mesic: 9000- 10,000 ft.*	2.4-3.6	20	2.4	1 layer, upper	Largest air spaces	20	660 \times 860	2-3 \times 6-8(1) 5 \times 8(2) 5-6 \times 9-10(3)	6-7 \times 9-12(1) 7-9 \times 14-15(2) 9-13 \times 12-13(3)	In some cases, middle la- mella dis- solves and older phloem goes to pieces or is crushed	
Xeric: Laramie Range, 7000- 8000 ft.†	4.8-7.2	36	3.6-4.8	3-4 layers, up- per and lower	Air spaces but noticeably smaller than above	30	800 \times 800	5 \times 9-10(1) 5 \times 9-10(4)	8-10 \times 11-13(1) ? \times 9-11(4) (crushed cells cannot be counted)	In some cases, middle la- mella dis- solves and older phloem goes to pieces or is crushed	

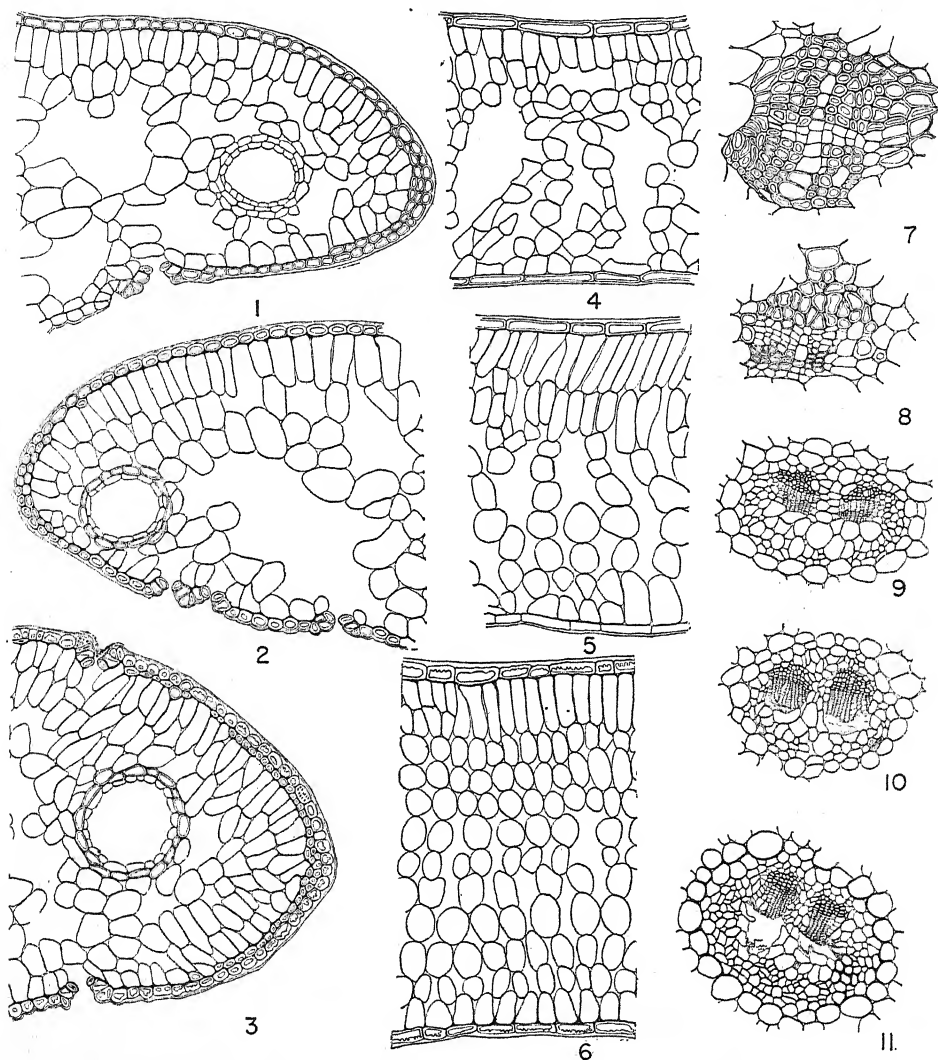
* Young leaves and leaves from

* Young leaves and leaves from mesic habitat lignified in xylem, slightly in pericycle and inner tangential walls of endodermis.
† Leaves from xeric habitat lignified in hypodermal cell walls, all of pericycle, xylem, ray between bundles, and inner tangential walls of endodermis.

‡ Numbers in parentheses indicate age in years.

mesic habitat and smallest in the stunted trees of the Krummholz. Counts of the cells of the xylem and phloem show some

In all leaves examined there were more phloem than xylem cells in the cross-sections, except in those in which the



FIGS. 1-11.—Needles of *Abies lasiocarpa*: Fig. 1, cross-section, 1 year, mesic habitat. Fig. 2, cross-section, 4 years, mesic. Fig. 3, cross-section, 4 years, xeric. Fig. 4, longisection, 1 year, mesic. Fig. 5, longisection, 4 years, mesic. Fig. 6, longisection, 4 years, xeric. Fig. 7, cross-section of vein, 3 years, lee side of Krummholz. Fig. 8, cross-section of vein, 1 year, mesic. Fig. 9, cross-section of stele, 1 year, mesic. Fig. 10, same, 4 years, mesic. Fig. 11, same, 4 years, xeric.

increase in the number of cells in both tissues after the first year. The greatest increase in number of conducting cells is in the leaves from the mesic habitats.

oldest phloem was crushed and apparently dissolved. The phloem cells from younger leaves and leaves of mesic habitats have the thickest walls. The cam-

bium is evident, even in the oldest needles, for it remains as an area of thinner-walled cells between the xylem and phloem, at least during July and August, when these leaves were collected. Figures 10 and 11 show cross-sections of veins from needles from mesic and xeric habitats. The confirming evidence that there is cambium growth is the actual increase in the number of cells of the xylem and phloem, rather than the appearance of the cells.

The oldest needles found on the trees in the xeric habitat were 12 years old, while those in the mesic habitats remained on the trees as long as 17 years. The oldest needles on the lee side of the Krummholz were 12-16 years old, while the oldest on the windward side were 9-12 years. The cuticle is thinner on the leaves of the windward side, the hypodermal cell walls tend to be less thickened, the air spaces as seen in the longitudinal sections are in general larger

TABLE 3
PICEA ENGELMANNI, SIZE OF LEAVES

HABITAT	ONE YEAR OLD	TWO YEARS OLD	THREE YEARS OLD	FOUR YEARS OLD	AV. SIZE FOR FOUR YEARS
	Length×Width×Thickness (mm.)	Length×Width×Thickness (mm.)	Length×Width×Thickness (mm.)	Length×Width×Thickness (mm.)	Length×Width×Thickness (mm.)
Alpine: Krummholz, windward side, 11,000 ft.	17 ×1.3 ×1.2	19 ×1.3 ×1.1	17 ×1.2 ×1.0	17 ×1.3 ×1.0	17.5×1.3×1.1
Alpine: Krummholz, lee side, 11,000 ft.	20 ×1.3 ×1.1	24 ×1.3 ×1.15	23 ×1.2 ×1.1	20 ×1.4×1.2	21.3×1.3×1.1
Mesic: near stream, 9000-10,000 ft.*	19.8×1.5 ×1.0	21.8×1.25×0.98	21.7×1.26×0.86	18.7×1.0×0.95	20.5×1.2×0.95
Xeric: high on Laramie Range (Veedavoo Rocks)	15.4×1.25×1.26	15.4×1.3 ×1.34	16.0×1.34×1.2	14.0×1.3×1.1	15.2×1.3×1.2

* Largest needles (23×1.7×1.3) found in mesic habitat.

PICEA ENGELMANNI (PARRY) ENGELM.

The needles of spruce in xeric and mesic habitats vary less than the fir needles. They are never flattened but always four-angled in transverse section. The leaves growing in the xeric habitats are shorter, generally thicker, and much more rigid than those in the mesic situation (table 3). The needles of the spruce of the Krummholz do not have the same relative sizes as do the fir needles. The spruce needles are longer and somewhat thicker on the lee side and shorter and somewhat thinner on the windward side. There is a tendency, even in the spruce, for the needles on the windward side to be somewhat more mesomorphic in internal structure than the needles on the protected or lee side (table 4).

than those in the leaves from the lee side, and the veins are smaller in cross-section. These comparisons indicate that these leaves are more nearly like those of mesic habitats. There is more lignification in the leaves on the windward side.

In the habitats below the alpine Krummholz, the greatest thickness of cuticle is in the drier habitats. There is less variation in the depth of the stomatal pits than in the alpine fir. Many of the stomatal pits in the leaves from the xeric habitats are filled with an amorphous material (fig. 14). These deposits are loosened in the preparation of microscopic slides and were found to be directly connected with the outermost layer of the cuticle. The epidermal and hypodermal cells are noticeably thicker-walled in the needles from the xeric habi-

TABLE 4

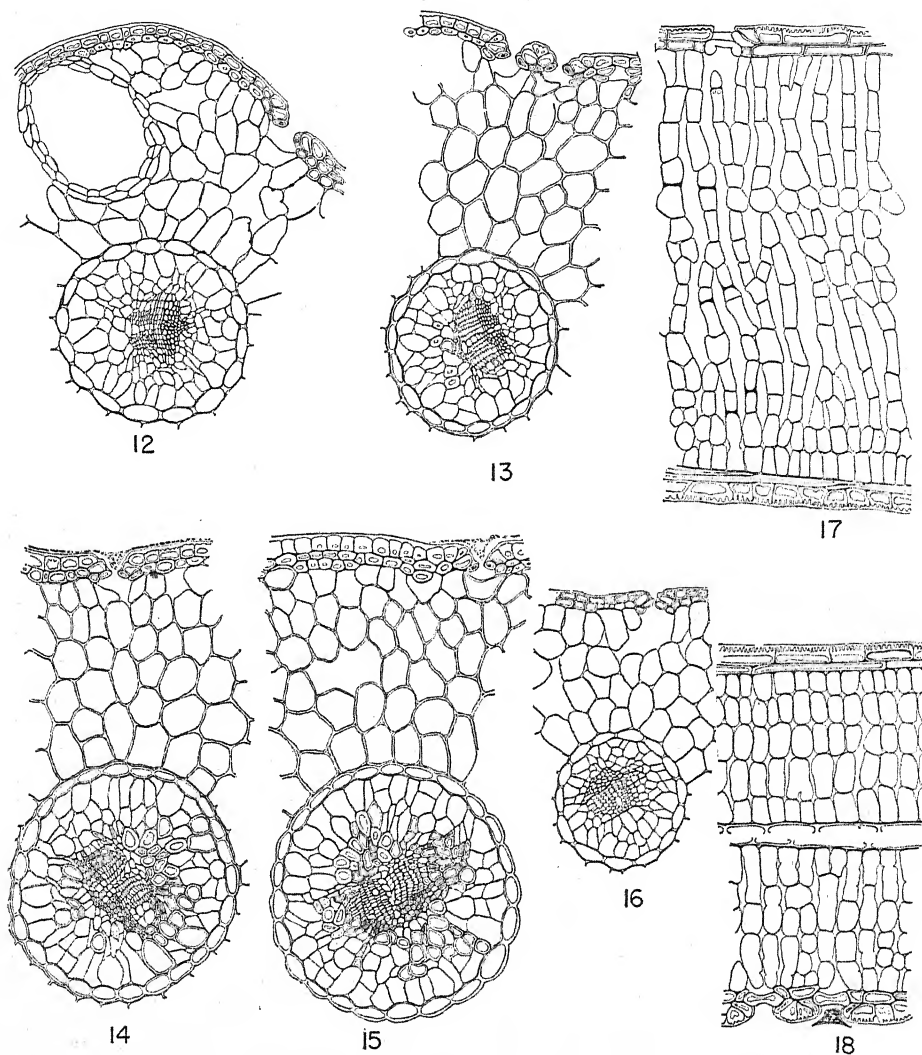
PICEA ENGELMANNI, INTERNAL STRUCTURE OF LEAVES

HABITAT	AV. THICK- NESS OF CUTICLE (μ)	AV. DEPTH OF STOMATAL PIT (μ)	AV. THICKNESS OF HYPODERMAL CELL WALLS (μ)	GREEN MESOPHYLL, PALISADE-LIKE CELLS IN LONGISECTION	APPROX. SIZE (AV- ERAGE) OF CROSS-SEC- TION OF VEIN (μ)	AV. NUMBER OF XYLEM CELLS		REMARKS
						Thickness X	Width	
Alpine: Krummholz, windward side, 11,000 ft.	3.2-7.2	25-40	2.4-3.2(1)* 3.2-7.2(2) 3.2-7.2(3)	All cells somewhat palisade-like, larger air spaces than on lee side	105 X 160	4-5 X 12-15	10-12 X 16-19(1) 10-14 X 12-18(2) (outer cells crushed)	Greater lignification than on lee side
Alpine: Krummholz, lee side, 11,000 ft.	4.8-9.6	25-40	2.4-3.2(1) 3.2-8.4(2) 4.8-8.4(3)	Similar to above but more com- pact, with smaller air spaces	125 X 165	4-5 X 12-15	9-10 X 15-16(1) 10-15 X 15-20(2) (outer cells crushed)
Mesic: 9000-10,000 ft.	2.4-7.2	25-30	2.4-4.8(1) 4.8-7.2+(4)	Open, with large spaces	75 X 62	5 X 12	9-10 X 11-15 (outer cells crushed; cam- bium not evi- dent in older needles)	Lignified in all cells but phloem and epithelial of resin ducts; lignifica- tion mainly in middle lamellae
Xeric: Laramie Range	7.2 \pm	30+ (many filled with homogene- ous sub- stance)	4.8-9.6	Air spaces rela- tively small, cell walls thickened and somewhat lignified	90 X 150	3-4 X 12-13	13-17 X 12-20	Lignified in all cells of peri- cycle and endodermis, xylem, hypodermis, tan- genial walls of epider- mis, and middle lamel- lae of mesophyll cells
Alpine: on mountain- side, over 11,000 ft. Current-year needles	3.2 3.2 4.8	10-15	1.2-2.4	Very open	75 X 90	2-3 X 9-10	6-7 X 9-11	Percycle lignified but not endodermis, except slightly in inner tangen- tial and radial walls; hy- podermis, epidermis, and mesophyll walls
One year needles	4.8	15-25	Up to 4.8	Palisade-like cells in rows with spaces between	85 X 110	3-4 X 8-11	10-11 X 11-12	slightly lignified

* Numbers in parentheses indicate age in years.

tats, and the intercellular spaces become filled by cell wall substances. In the mesic situations there are air spaces be-

renchyma of spruce, as in the fir, are much more evident in the longitudinal sections (fig. 17). The air spaces vary



FIGS. 12-18.—Needles of *Picea engelmanni*: Fig. 12, cross-section, 1 year, mesic habitat. Fig. 13, cross-section, 4 years, mesic. Fig. 14, cross-section, 1 year, xeric. Fig. 15, cross-section, 4 years, xeric. Fig. 16, cross-section, 1 year, high alpine. Fig. 17, longisection, 1 year, mesic. Fig. 18, longisection, 4 years, xeric.

tween many of the hypodermal cells (fig. 13). The resin ducts in the thicker leaves of xeric habitats are larger in diameter than those in the mesic habitats. The intercellular spaces in the chlo-

with the habitat, as in the fir, and are larger in the leaves from a mesic habitat. The appearance of a slight infolding in these mesophyll cells of Englemann spruce is made by barlike thickenings on

the radial walls between areas of simple pits. These thickenings do not produce the true infoldings characteristic of the pines, except in a few of the mesophyll cells.

The size of the cross-section of the vein is greatest in the leaves from xeric habitats. The phloem has a greater number of cells than the xylem, and the phloem increases more than the xylem by the growth of the cambium. The evidence for cambium growth is also an actual increase in the number of cells of

Thickness of the cuticle does not vary as much as on the leaves of the alpine fir. There is a sloughing off of the outer layer (or layers) of cuticle from the older needles and from those of a xeric habitat. The amount of this sloughing was not determined. There was no evidence of this loss of cuticle in the fir but some evidence that it occurs in the spruce. The stomatal pit is overarched by the subsidiary cells of the guard cells more than by the cuticle. The depth of the stomatal pit is practically the same in all habitats.

TABLE 5
PINUS CONTORTA, SIZE OF LEAVES

HABITAT	ONE YEAR OLD	TWO YEARS OLD	THREE YEARS OLD	FOUR YEARS OLD	AV. SIZE FOR FOUR YEARS
	Length×Width×Thickness (mm.)	Length×Width×Thickness (mm.)	Length×Width×Thickness (mm.)	Length×Width×Thickness (mm.)	Length×Width×Thickness (mm.)
Alpine: near Lake Marie and Medicine Bow Mt., about 11,000 ft.	38 ×1.45×0.75	44 ×1.5×0.8	40×1.4×0.7	41 ×1.4 ×0.7	40.7×1.4×0.74
Mesic: near Univ. of Wyoming Science Camp, 9000-10,000 ft.	47.5×1.6 ×0.8	51.2×1.6×0.6	62×1.7×0.87	50 ×1.7 ×0.8	52.7×1.6×0.77
Xeric: dry rocks at the Vedavoo Rocks on Laramie Range	36 ×1.36×0.65	39.7×1.4×0.66	40×1.8×0.66	35.2×1.38×0.69	37.7×1.48×0.66

the xylem and phloem. The cambium is not so active as it is in the alpine fir. Lignification is greatest in the xeric habitats, and all cells of the stele are completely lignified, except the phloem—which was never lignified in the leaves examined.

PINUS CONTORTA LOUD.

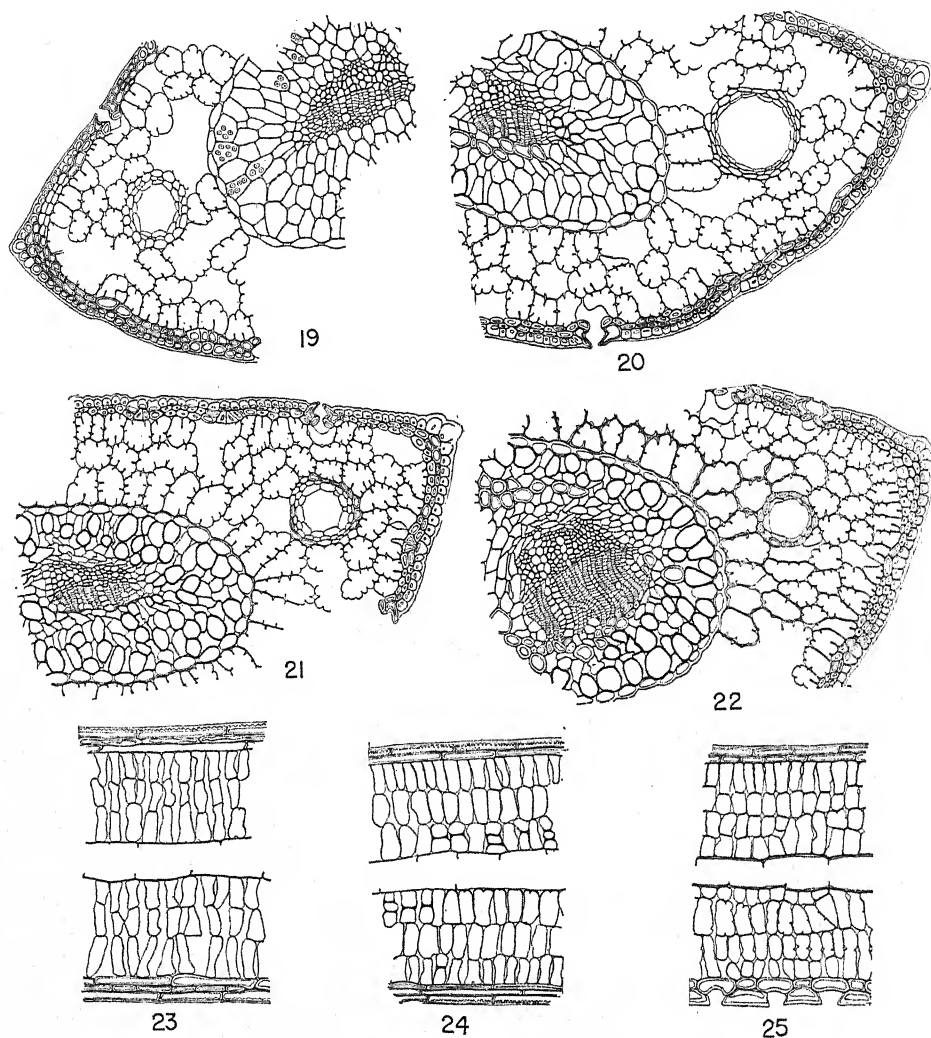
The needles of this pine vary much in size in different years, and there is a noticeable variation in the extremes of habitat. The needles from trees in the most xeric habitat are smallest in all dimensions; those of trees at the highest altitude for the species in this area are next in size; and trees growing in the most mesic habitats have the needles largest in all dimensions (table 5).

The thickness of the epidermal cell walls increases with the age of the needles as in other conifers, the outer walls becoming thicker than the inner. These thickenings are variable in form because of the deep simple pits. There are also simple pits in the hypodermal cell walls, and the walls of these cells become thicker and thicker, until the lumen of the cells may be extremely small in the oldest leaves and in those from a xeric habitat.

The green mesophyll cells in all habitats are infolded. There is an actual infolding of the radial and tangential walls. Longitudinal sections show that these infoldings are of the whole wall and include both primary and secondary walls. Figures 19 and 20 show the degree of in-

folding, the character of the infolded wall, and the greater thickness of the mesophyll cell walls in the older leaves

spaces of greater size than is evident in the cross-sections. As in other species, the largest intercellular spaces



FIGS. 19-25.—Needles of *Pinus contorta*: Fig. 19, cross-section, 1 year, mesic habitat. Fig. 20, cross-section, 8 years, mesic. Fig. 21, cross-section, 1 year, xeric. Fig. 22, cross-section, 8 years, xeric. Fig. 23, longitudinal section from mesophyll, 1 year, mesic. Fig. 24, same, 4 years, mesic. Fig. 25, same, 8 years, xeric.

and in those from the xeric habitats. Although cross-sections of needles from xeric habitats show only very small air spaces, the longitudinal sections of xeromorphic forms show that there are air

are in the leaves from the mesic habitats (figs. 23, 25).

The endodermis is complete and without passage cells. The greatest number of fiber cells occurs in the pericycle of

the leaves that grew in the xeric habitats (fig. 22). The two veins each increase in size after the first year in both extremes of habitat, as shown by the approximate numbers of cells in the xylem and phloem (table 6; figs. 20, 22). This increase in size of the veins is from cambium. The

the inner walls of the epidermis become completely lignified. In the green mesophyll the middle lamella, primary wall, and some of the secondary wall are lignified, and near the hypodermis and adjacent to the endodermis the secondary wall becomes completely lignified. The

TABLE 6
PINUS CONTORTA, INTERNAL STRUCTURE OF LEAVES

HABITAT	AV. THICK- NESS OF CUTICLE (μ)	AV. DEPTH OF STO- MATAL PIT (μ)	AV. THICK- NESS OF WALLS OF HYPODERMAL CELLS (μ)	MESOPHYLL IN LONGISECTION (CHLORENCYMA)	AV. NUMBER OF XYLEM CELLS	APPROX. NO. OF PHLOEM CELLS
					Thickness \times Width	Thickness \times Width
Mesic: needles full- grown but not year old	2.4	25	1.2	Thin-walled, resin duct walls not thickened, spaces evident	4-5 \times 17-18	6 \times 20
Mesic: needles 5 years old	4.8	25	1.2-3.2	Spaces evident	10 \times 24	20 \times 25*
Mesic: needles 8 years old	4.8	25	1.2-3.2	Cell walls thick- ened, middle lamellae ligni- fied, spaces evi- dent	7 \times 20	22 \times 20*
Xeric: needles full- grown but not year old	3.2	25	1.2-3.2	Walls slightly thickened, spaces evident	6 \times 17	15-17 \times 16-17*
Xeric: needles 4 years old	4.8	25	2.4-4.8	Intermediate	7 \times 17	17 \times 16*
Xeric: needles 8 years old	4.8-9.6 (at edges of needle)	25	1.2-3.2+	Compact and thick-walled, walls lignified, compact in both trans- and longi- section	7 \times 20	22 \times 21*

* Some cells crushed.

xylem of the needles from the xeric habitat increases by the addition of only a few cells; but the phloem shows a marked increase in number of cells, and the older phloem cells are crushed by this growth. In the leaves from the mesic habitats the xylem increases more than in those from the xeric habitats.

Lignification is greatest in the older needles and in the needles from the xeric habitats. In the leaves from the xeric habitats all cell walls of the pericycle, xylem, the endodermis, hypodermis, and

cells of the resin ducts are lignified—except the epithelial cells. The leaves of mesic habitats show lignification first in the middle lamellae and primary walls, then only in narrow lamellae within the secondary wall in the oldest leaves examined.

Discussion

The cuticle of the leaves of these three species is thickest in the alpine Krummholz and xeric habitats, but in the alpine Krummholz the internal structure of the

leaf is more nearly like that of mesomorphic leaves. In the plants of the alpine meadows around the Krummholz the cuticle is very much thickened also, although the mesophyll of such leaves has relatively very large air spaces, *Polemonium* sp. even having the open mesophyll of an aquatic leaf. The buckbean growing in water at an altitude of 11,000 feet has a surprisingly thick cuticle and the mesophyll of a typical floating leaf. From these observations, it seems probable that the thickness of cuticle varies with the light intensity.

This study did not include any measured data on the physical features of the habitat, or any studies on the relative amounts of water lost. It raises interesting questions, however, that only such data can answer. Why is there a difference, for example, in the thickness of cuticle and internal structure in leaves on opposite sides of the Krummholz, with the more mesomorphic structure occurring in leaves of the windward side?

WEAVER and MORGENSEN (18) measured the water lost from conifer seedlings and found that *Pinus ponderosa*, *Pseudotsuga mucronata*, and *Picea engelmanni* lost more water than the seedlings of *Acer glabrum* and *A. saccharinum* per square decimeter of leaf surface. These seedlings grew in the same environment during the time the measurements were made. They also found that the greatest loss of water per day was from 3-year-old seedlings of *Pinus ponderosa* and *P. banksiana*. The 3-year-old seedlings of *Abies grandis* and Douglas fir lost less water per day than the pines. The loss of water from *Picea engelmanni* was less than from the pines but more than that lost by the white fir and Douglas fir.

The lodge-pole pine leaves are the most xeromorphic of the three trees of this study and are similar in general

structure to the pines just mentioned. The alpine fir is the most mesomorphic and has leaves most nearly like those of the white fir and Douglas fir used by WEAVER and MORGENSEN. The spruce is the same species. From these data, and from the work of TURRELL (17) showing that leaves with the smallest intercellular spaces lose more water per unit of epidermal surface than leaves with large intercellular spaces, it is to be expected that greatest water loss does occur from the leaves of *Pinus contorta*. Does it then follow that the older leaves of these conifers lose more water than the young leaves and that the trees growing in xeric habitats lose more water per unit of epidermal surface than the trees from the more mesic habitats?

Since abscission of the needles of trees in xeric habitats occurs 5-7 years sooner than from trees in mesic habitats, the total amount of water lost must be thereby decreased in trees of xeric habitats. This may therefore be a factor in explaining the continued growth of certain conifers in relatively dry habitats.

It has been generally assumed that the xeromorphic structures described in these leaves from xeric habitats reduce water loss and have a survival value for the plant. The writer is citing these data on water loss measured in relation to intercellular space as a caution not to conclude that the compactness of mesophyll and the other structures typical of plants in xeric habitats are the factors enabling them to survive in those habitats.

Summary

1. In the mesic habitats of the two altitudes in the Medicine Bow Mountains investigated, the needles of *Abies lasiocarpa* were found to be longest, widest, and thinnest; those of *Picea engelmanni* longest and with the smallest

cross-section; and those of *Pinus contorta* larger in all dimensions than those from the xeric habitats of the Laramie Range.

2. Thickness of the cuticle increases with age of the leaves; it is thickest in the xeric habitats and in the bright sunlight of the alpine habitat. Thickness of the walls of the epidermal cells varies in the same way.

3. The stomatal pits tend to be deeper in the older leaves and the leaves from xeric habitats on the fir and spruce; there is little variation in their depth on the pine.

4. The number of cells in the hypodermal sclerenchyma is greater in older leaves, and the greatest number is found in the leaves from xeric habitats.

5. The cells of the mesophyll in all three species are more compact in the

older leaves and most compact and with the smallest intercellular spaces in the leaves of trees from xeric habitats. The thickest cell walls and their greatest lignification occur in the leaves from xeric habitats. The resin ducts of largest diameter were found in the leaves from xeric habitats.

6. There is an increase in the size of the veins and in the number of cells of the veins in each species by the growth of cambium. The greatest increase in number occurs in the phloem. Most cambium growth is in the pine.

7. The oldest leaves are found in the trees growing in the mesic habitats. The oldest needles on the wind-blown trees of the high alpine flats are on their lee side.

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ZONAL STRUCTURE OF THE SHOOT APEX IN ENCEPHALARTOS, BOWENIA, AND MACROZAMIA¹

MARION A. JOHNSON

Introduction

A recent series of cyto-histogenetic studies on *Ginkgo*, *Cycas*, *Dioon*, and *Microcycas* by FOSTER (1, 2, 3, 4, 5) and on *Zamia* by JOHNSON (6) has brought to light a zonal type of meristem heretofore unsuspected. The feature common to these forms is a centrally placed group of cells which has been derived from the superficial layer and its derivatives and which in turn gives rise to a rib meristem and a mantle of flanking meristem. Except for certain variations, to be noted later, this is the fundamental type of architecture observed to date in the shoot apex of cycads. The present paper, while strictly preliminary in scope, owing to the relatively small amount of available material, describes the shoot apex in *Encephalartos*, *Bowenia*, and *Macrozamia*.

MATERIAL AND METHODS.—Special thanks are due Mr. E. R. Thorp of the Botanic Garden, Durban, South Africa; Mr. F. R. Long, Superintendent of Parks, Port Elizabeth, South Africa; and Mr. H. Herre, University of Stellenbosch, Stellenbosch, South Africa, who generously provided Rutgers University with a collection of South African cycads. The following were sacrificed from among the duplicates for histological study: *Encephalartos altensteinii* Lehm.—dormant specimen with caudex 4 cm. in diameter; *E. woodii* Hort.—dormant plant with caudex 11 cm. in diameter; *E. horridus* Lehm.—plant with six new leaves and caudex 28 cm. high and 18 cm. in diameter; *E. lehmanii* Lehm.—bud with one leaf; *E. villosus* Lehm.—plant with trunk 30 cm. high.

¹ Contribution from the Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey.

The late Dr. C. J. Chamberlain kindly placed two seedlings of *Encephalartos frederici-guilielmi* Lehm., three seedlings of *Bowenia serrulata* (Andre) Chamberlain, and three embryos of *Macrozamia moorei* F. Muell. at my disposal. My colleague, Professor M. A. Chrysler, generously contributed the following specimens which had been collected for him by Professor A. A. Lawson in Queensland, Australia: *Bowenia serrulata*, seedling and conebearing female plant with caudex 10 cm. in diameter from which five apices were obtained; *Macrozamia spiralis* Miq., two seedlings with caudex 12–15 mm. in diameter; and *M. corallipes* H., plant with stem 10 cm. in diameter.

The shoot apices were carefully dissected from the surrounding leaf bases of the living specimens and fixed in formalin-acetic-alcohol. All material was dehydrated in tertiary butyl-alcohol, imbedded in paraffin, sectioned at 8 μ , and stained with either tannic acid and iron chloride or safranin and Ehrlich's haematoxylin.

The large size of the shoot apex has made it exceedingly difficult to recognize median longisections with certainty, so that when the term "median" is used it is to be understood that such sections are only approximately median.

Observations

ENCEPHALARTOS

The shoot apex in *Encephalartos* is deeply sunken within a protective armor of cataphylls and leaf bases. It is either broadly cone-shaped or moundlike and may terminate in a plateau about a dozen cells in diameter (figs. 1–5). Measurements of tips taken from median

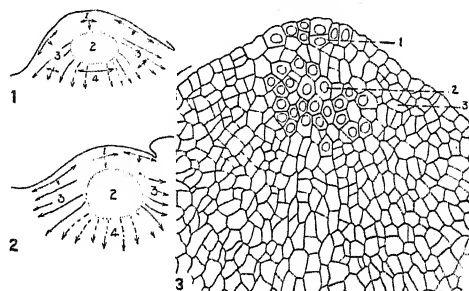
longisections are: *E. villosus*, 1263 μ in diameter 343 μ from the apex; *E. woodii*, 1170 μ in diameter 436 μ from the apex; *E. horridus*, 858 μ in diameter 171 μ from the apex; *E. lehmanii*, 468 μ in diameter 171 μ from the apex; and *E. frederici-guilielmi*, 530 μ in diameter 187 μ from the apex. The last two measurements are from a bud and seedling, respectively, and consequently give little indication of the size to be expected in adult plants. The others surpass the dimensions recorded for the Florida species of *Zamia* but are considerably smaller than those given by FOSTER (3, 4, 5) for *Cycas revoluta* (2–3.2 mm.), *Dioon edule* (1.6–1.9 mm.), and *Microcycas calocoma* (0.5–2 mm.). It seems likely that greater dimensions will be found in specimens of *Encephalartos* with massive trunks, and possibly a 10-foot specimen of *E. woodii* would have a shoot apex approaching that of *C. revoluta* in size.

The shoot apex consists of four zones (figs. 1, 2).

ZONE 1.—The summit of the shoot apex is occupied by a zone of initiation which is generally two to six cells deep and some six to twenty cells in diameter. The superficial layer divides by the insertion of both anticlinal and periclinal walls, thereby contributing to surface growth as well as to the deeper layers within the apex. The latter either build up short chains of cells or complicate the cell pattern by anticlinal and periclinal divisions (figs. 3–5). In dormant tips, the superficial layer may contain conspicuous vacuoles (fig. 4).

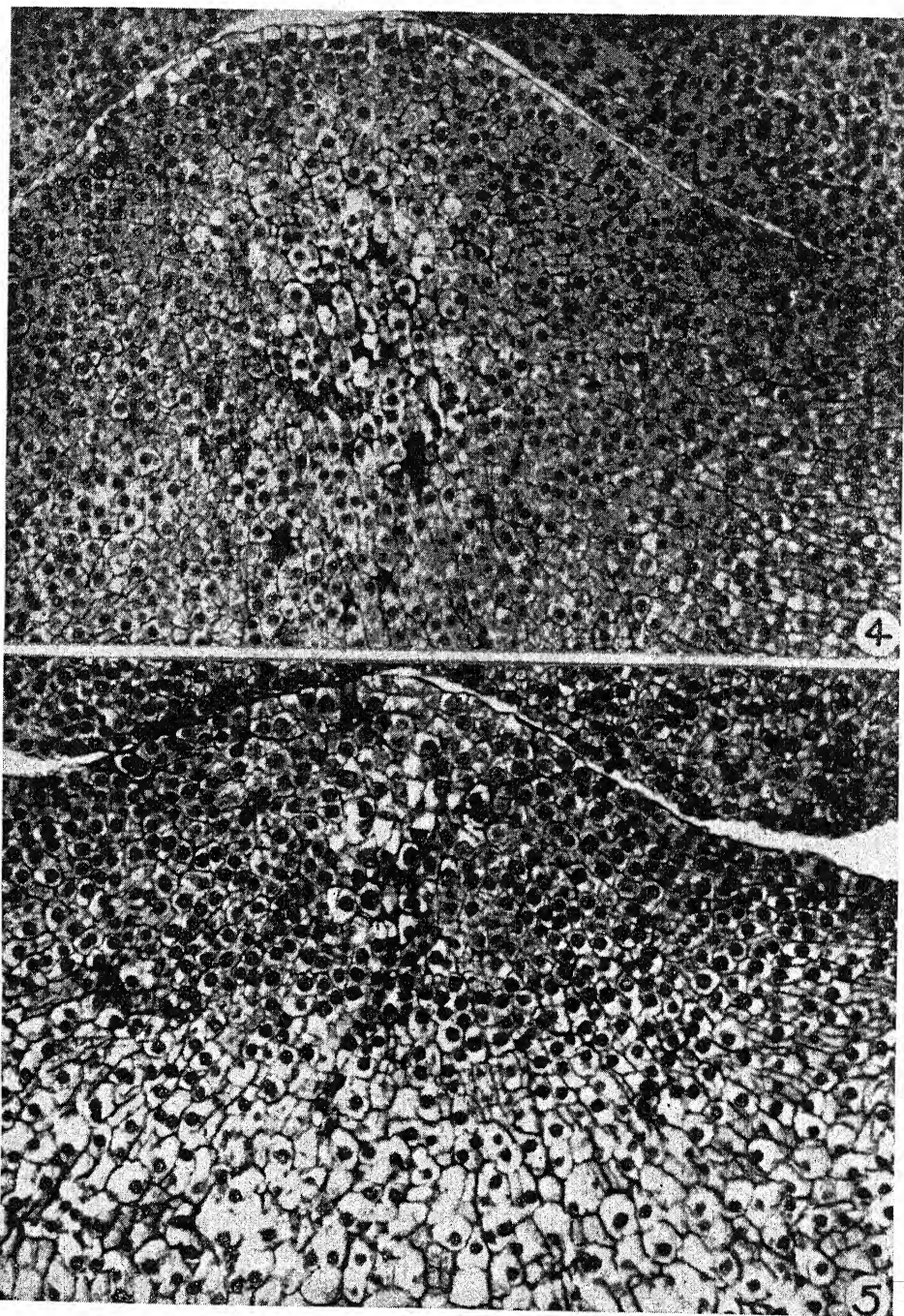
ZONE 2 (CENTRAL MOTHER CELLS).—The zone of initiation is underlaid by an area roughly spherical in shape and readily characterized by cellular enlargement and a complex cell pattern. This zone contrasts sharply with the

more dense protoplasm and regular alignment of the cells radiating from it (divergent growth). These cells, owing to their position and genetic relationship in the shoot apex, have been appropriately designated the central mother cells by FOSTER (1, 4). They originate from the zone of initiation (convergent growth), and—since the transition between the two zones may be gradual—they are not always clearly delimited. In



FIGS. 1-3.—Figs. 1, 2, diagrams showing zonal structure in shoot apex of *E. woodii* and *E. horridus*, respectively. Zone 1 is region of initiation which through periclinal and anticlinal divisions builds up zone 2 (central mother cells). Some contribution is also made to the peripheral meristem (zone 3). Divergent growth from the zone of central mother cells gives rise to the rib meristem (zone 4) and the remainder of peripheral meristem, except for outer part which is derived from the superficial layer. Arrows indicate direction of growth. Fig. 3, median longisection through apex of *E. frederici-guilielmi* (1, zone of initiation; 2, central mother cell zone; 3, peripheral zone). $\times 215$.

massive apices they are more deeply situated than in small ones (figs. 4, 5). It was impossible to determine whether this character is associated with species differentiation or with the size of the apex. The central mother cells are readily plasmolyzed by formalin-acetic-alcohol and are thus rendered conspicuous in stained preparations. Cell division is at random, and intricate cell complexes are built up, giving the region the appearance of a "massiges Meristem" (7). Genetically related cells in the form of



FIGS. 4, 5.—Fig. 4, *E. lehmannii*: median longisection through shoot apex. Central mother cells show tendency to appear in filaments still inclosed in original mother cell wall; wall thickenings conspicuous; convergent growth toward and divergent growth from zone of central mother cells. $\times 155$. Fig. 5, *E. villosus*, median longisection through shoot apex. Note prominent vacuoles in central mother cells. $\times 122$.

files or irregular blocks are often inclosed in the wall of the original mother cell. Conspicuous wall thickenings are common, especially at the points where several cells touch.

ZONE 3 (PERIPHERAL MERISTEM).—The central mother cells in all the apices examined, regardless of size or species, were imbedded in active meristematic tissue which radiated from them in long files. For the most part these have had their origin in periclinal, anticlinal, and oblique divisions at the edge of the zone of central mother cells. The outer part of the peripheral meristem has been derived from the zone of initiation and the superficial layer and may consist of rows of cells or a complicated irregular pattern. The inner part of this zone is derived from the central mother cells. The greatest activity occurs at the base of the apex, where the leaf primordia originate and where the characteristic cellular arrangement is in files parallel to the surface.

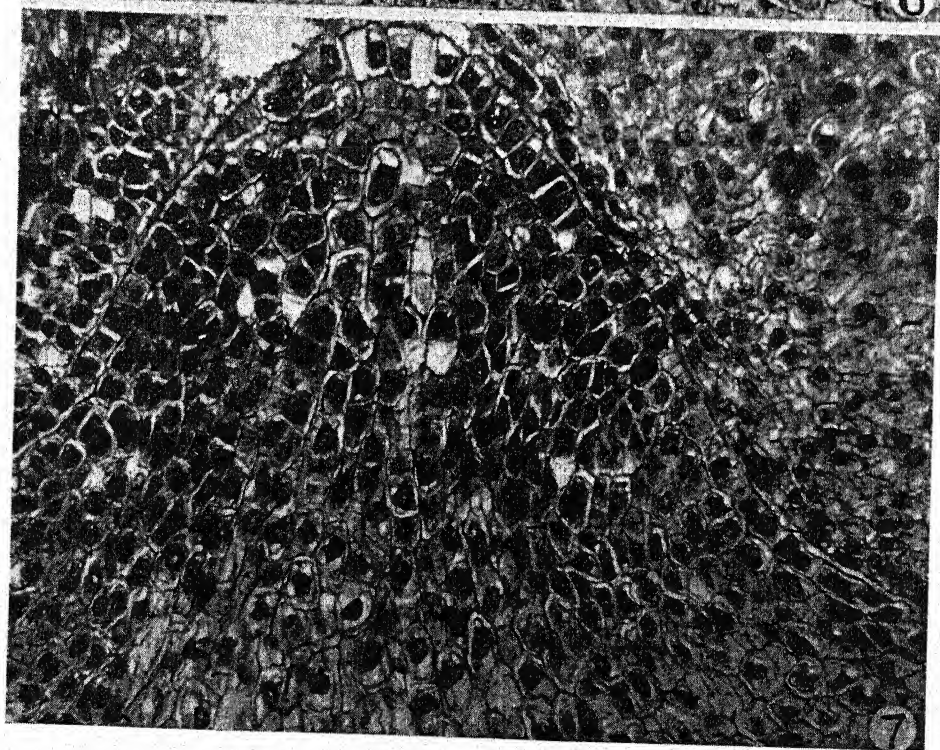
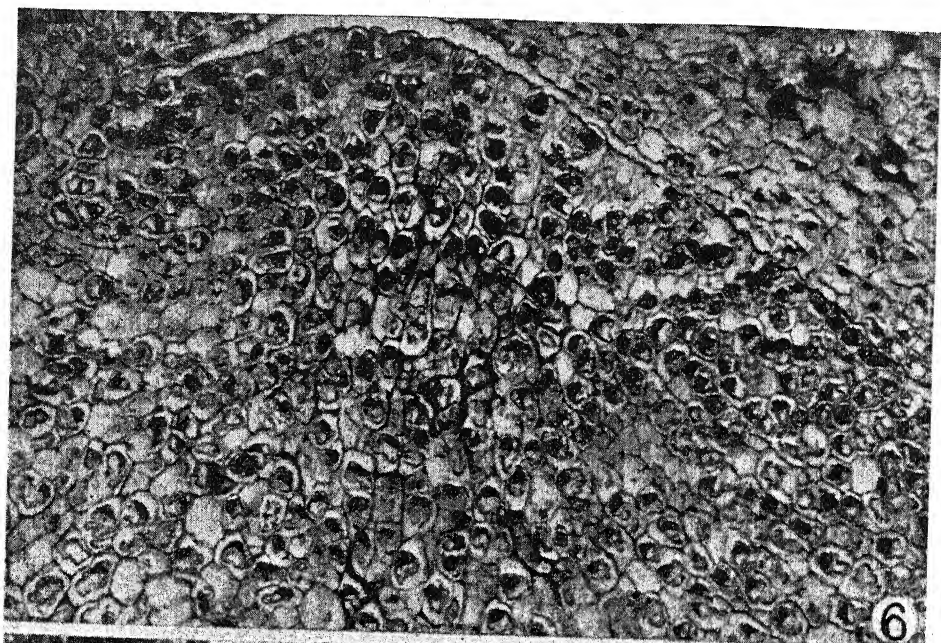
Histologists early called attention to the fact that the shoot apex in cycads is at the bottom of a depression in the stem tip. This condition is largely brought about by a reorientation of division figures and subsequent growth in the peripheral meristem beneath the leaf bases. Divisions parallel to the long files produce a series of cambium-like cells which elevate the periphery of the stem and the leaf bases above the shoot apex. Cortex, vascular tissue, and perhaps some of the pith, mature from the peripheral meristem.

ZONE 4 (RIB MERISTEM).—The long files of cells derived from the base of the central mother cell zone represent a typical rib meristem from which the massive pith differentiates. In the upper portion the cells are densely protoplasmic and division is principally in the

transverse plane. Below this level vacuolation becomes noticeable and new walls appear in all planes. The pith now begins an enormous increase in volume, due to both cell division and cell enlargement. The depth of the rib meristem shows some variation, which may possibly be associated with age and periodic growth of the shoot apex. In the seedling of *E. frederici-guilielmi*, elongation of the axis seems to be greater than increase in thickness, and the pith cells are longer than in the other species; also the rib meristem is inconspicuous (fig. 3) and approaches the condition observed by FOSTER (2) in the seedling of *Cycas revoluta*. Whether this condition persists in the adult plant, as it does in *C. revoluta*, cannot be determined until older material is available.

BOWENIA

These observations are based upon longitudinal sections cut from seven apices. The shoot apex is moundlike in shape (fig. 6) and closely invested by several leaf bases. The largest apex measured 1248 μ in diameter 200 μ from the summit. The initiation, central mother cell, peripheral and rib meristem zones are clearly marked and are similar to those described for *Encephalartos* (fig. 6). The central mother cell zone is well developed in seedlings. One specimen, bearing three young leaves and having an axis 10 mm. in diameter, had a shoot apex 545 μ in diameter 45 μ from the tip and a central mother cell zone which was spherical in shape and measured 300 μ in diameter. The contained cells were highly vacuolate and were arranged in a complicated pattern. This pattern seems to be resolved to vertical files of cells in larger apices (fig. 6). The superficial layer contributes less to the pe-



FIGS. 6, 7.—Fig. 6, *Bowenia serrulata*, median longisection showing zonation in shoot apex. $\times 210$.
 Fig. 7, *Macrozamia spiralis*, seedling apex showing tendency for cells to be arranged in vertical files.
 Zone of central mother cells poorly developed in seedlings. $\times 235$.

ripheral meristem in seedlings than in older plants.

MACROZAMIA

LAWSON considered the two seedlings studied in this investigation to be *Macrozamia spiralis* (fig. 7), while the large plant (fig. 9) was identified as *M. corallipes*. The latter species is listed as a synonym of *M. spiralis* in Index Kewensis, and according to this opinion the description which follows is therefore for *M. spiralis*. The shoot apex is conical in shape and in the large specimen (fig. 9) measured $624\ \mu$ in diameter $265\ \mu$ from the tip. The zonation previously observed in cycads is repeated in the shoot apex of *Macrozamia* (fig. 9). It is noticeable, however, that many of the cells tend to be disposed in files parallel to the steep sides of the shoot apex, and while those of the rib and peripheral meristems diverge from the central mother cell zone, yet the angle of divergence is much less than in *Bowenia* or *Encephalartos*. This condition is further emphasized in seedlings (fig. 7) and embryos (fig. 8), where a relatively small group of subapical initials serves as the ultimate origin for all cells except those derived from the superficial layer.

Attention should be called to the enormous shoot apex in the embryo of *M. moorei*, which in the material studied (fig. 8) measured $625\ \mu$ in diameter $250\ \mu$ from the tip. This is far larger than anything seen even in seedlings of *Cycas revoluta* or *Microcycas calocoma* by FOSTER (2, 5) or of *Zamia* by JOHNSON (6).

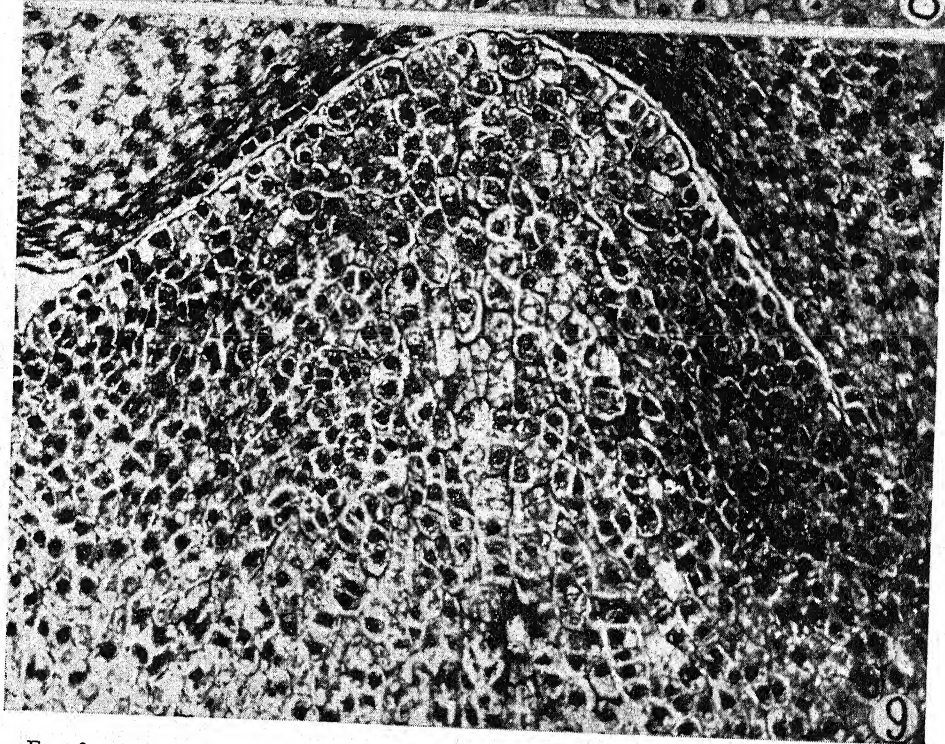
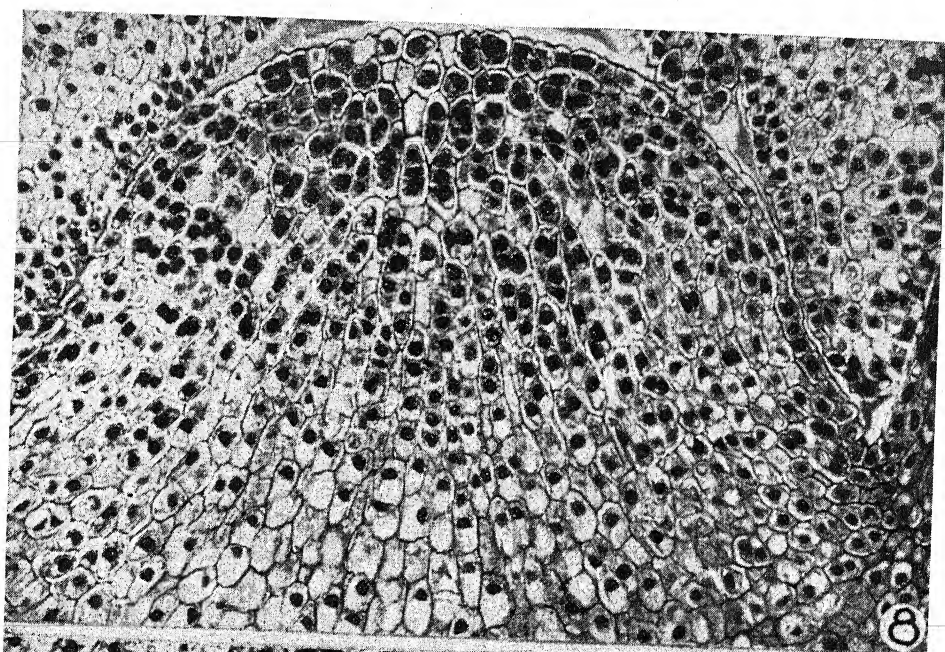
Discussion

The zonal structure of the shoot apex, as originally described by FOSTER (2) for *Cycas revoluta*, is now known to occur in *Dioon*, *Microcycas*, *Zamia*, *Encephalartos*, *Bowenia*, and *Macrozamia*. In

addition, all have a superficial layer which divides in both periclinal and anticlinal planes; hence cannot be considered as a protoderm. Further agreement is found in the absence of an apical cell. The summit of the shoot apex is occupied by a complex of initials whose number varies with the dimensions of the apex. Those at the exact geometrical center can be compared with a master apical initial only in that they remain meristematic throughout the life of the shoot apex.

The concept of the architecture of the shoot apex in the cycads will not be complete until *Ceratozamia* and *Stangeria*, as well as seedlings from all the genera, have been thoroughly investigated. Our knowledge to date, from seven of the genera, may be briefly summarized as follows. The initial zone is composed of actively dividing cells which converge to the zone of central mother cells. Divisions are anticlinal, periclinal, and oblique. The central mother cell zone, with its generally conspicuously vacuolate cells arranged in a complex pattern, is a region of increase in volume and upon "rejuvenation" at the periphery produces divergent files of cells. The zone of peripheral meristem has a dual origin, in that the outer portion is derived from the superficial layer, while the inner part is from the central mother cells. Leaf primordia, cortex, vascular tissue, and perhaps some of the pith are derived from this zone. A region of rib meristem separates the central mother cells from the pith, except in *Cycas*. Here the demarcation between the maturing pith and the central mother cells is indistinct.

There is a tendency in the seedling of *Macrozamia spiralis* for the cellular arrangement to be in long files parallel to the steep sides of the conical apex. This feature, together with the small number



FIGS. 8, 9.—Median longisections: Fig. 8, *M. moorei*, through apex of embryo; note absence of central mother cell zone and divergent growth from zone of initiation. $\times 185$. Fig. 9, *M. spiralis*, showing zonal structure in shoot apex of adult plant. $\times 172$.

of subapical cells in the initiation zone, is somewhat suggestive of the condition seen in many of the conifers. Further work is necessary, however, before a comparison can be attempted with any other species.

Summary

1. The structure of the shoot apex is described for six species of *Encephalartos* and for one species each of *Bowenia* and *Macrozamia*.

2. The shoot apex consists of four zones. The summit is occupied by a zone of initiation from which growth con-

verges to a zone of central mother cells where increase in volume predominates. Growth is divergent from the central mother cell zone.

3. A peripheral zone, which is derived from the central mother cells and superficial layer, flanks the central mother cell zone. Leaf primordia, cortex, and vascular tissue are derived from this zone.

4. The zone of central mother cells is underlaid by a rib meristem which builds up the massive pith.

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EFFECTS OF IRON DEFICIENCY ON RESPIRATION OF SUNFLOWER PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 561

PAUL R. GLENISTER

Introduction

Iron forms a part of the prosthetic groups of several of the respiratory enzymes found in plant cells—peroxidase, catalase, the cytochromes, and perhaps also cytochrome oxidase. These enzymes have been discussed recently (4, 16).

SOMERS, GILBERT, and SHIVE (14), working with soybean, found the respiration of iron-deficient less than that of normal plants, as might be expected.

The present work deals with a series of tests in which the respiratory gradients (respiration rates of leaves at various nodes along the stem) of green and of chlorotic sunflower plants were measured to determine whether a depression of the respiration of the leaves of the iron-deficient plants occurred in all the leaves or whether the depression was limited, like the chlorosis, to the younger leaves.

To supplement this study of respiration, the iron gradients (percentages of iron in leaves at various nodes along the stem) of the green and chlorotic sunflower plants were measured to determine (a) the extent to which the leaves of the two kinds of plants differed in the amounts of iron they contained and (b) the manner in which the iron was distributed among the leaves. With such information, a relationship might be found between iron content, respiration rate, and chlorosis.

Material and methods

During the summer of 1942, sunflower plants (*Helianthus annuus* L.) were grown from seed from a commercial source. The seeds were first removed from the fruit coats and selected for uniformity. They were planted ten per crock in forty 2-gallon crocks which were filled with pure quartz sand. The sand had been cleaned with dilute acid and alkali solutions in the manner described by MINARIK and SHIVE (8) and had been washed thoroughly with distilled water. From the day of planting on, the crocks were supplied with nutrient solution (table 1). Half of them received a complete solution and the remainder received one to which no iron salt had been added. One liter of nutrient solution was supplied to each crock every morning. This was usually supplemented with one or more liters of distilled water given during the afternoon, depending on the heat of the day. Germination was complete in 3 days. The seedlings were thinned to one per crock after 2 weeks, in this way securing as uniform a set of plants as possible. Three such sets were grown, but not all the plants were used, because of attacks by aphids, mildew, and other agents.

The carbon-dioxide output of the

leaves was measured by using the method and apparatus of MULLISON (9). In this method the carbon dioxide produced by the plant material is determined by measuring the changes it causes in the electrical resistance of a definite amount of a solution of standard alkali kept in an absorption tower at a constant temperature. The leaves were inclosed in 500-ml. erlenmeyer flasks with three-holed rubber stoppers and kept there during the tests. One of the holes in the rubber stopper admitted an inlet tube which conducted air from a soda-lime trap to the

TABLE 1

Composition of complete nutrient solution
(iron salt omitted from minus-iron solution)

Components	Concentration
Ca(NO ₃) ₂	0.006 molar
KH ₂ PO ₄	0.0045
MgSO ₄	0.0045
B (in H ₃ BO ₃).....	0.5 p.p.m.
Mn (in MnCl ₂).....	0.25
Cu (in CuCl ₂).....	0.02
Zn (in ZnCl ₂).....	0.05
Fe (in FeC ₆ H ₅ O ₇).....	0.5

flask. The outlet tube passed through the second hole and led to the absorption tower. The ends of the inlet and outlet tubes were so placed in the flask that the air had to flow through the body of the flask (and thus over the leaf surface) in going from one tube to the other. The third hole was slit all the way to the side of the stopper, so that the stopper could be opened like a book and closed with the petiole of a leaf going through the third hole in it. A small amount of chewing gum effectively sealed the space between the petiole and the rubber. The flasks were supported by ring stands. By means of this method the simultaneous measurement of the carbon-dioxide output of ten intact leaves on a single plant could be made.

The leaves were placed in the flasks at

approximately 12:00 noon, and then kept in the dark during the entire subsequent periods. During the first 20 hours, air circulated through the flasks. Then followed a 4-hour period in which carbon-dioxide-free air passed through the flasks and the pipes of the apparatus. Next the absorption towers were put into the system and the carbon dioxide produced by the leaves collected over a 3-hour period. Fresh weights of the leaves were taken immediately after their removal from the apparatus. Dry weights were taken after the leaves had been in an oven kept at 97° C. for 24 hours.

The iron contents of individual leaves were measured by the official colorimetric method (1), using a ferric-alum solution as standard, as described by SNELL and SNELL (13).

Seventeen green and twenty chlorotic plants, possessing twelve to twenty-five leaves each, were tested for respiration gradients. Ten or eleven leaves per plant were tested for carbon-dioxide production; then all the leaves were analyzed for iron.

Results

Symptoms of iron deficiency were noticeable in the plants of the minus-iron treatment after the third week of growth. At this time the leaves of the fifth, sixth, and seventh nodes were pale green or yellow in color, with the principal veins of a darker green. No death of tissue occurred with the chlorosis. As these leaves matured they gradually assumed a greener color. The leaves which appeared at succeeding nodes behaved similarly; they developed as chlorotic leaves and became greener some time after expansion. As a result of this later greening of leaves originally chlorotic, the younger leaves were the only chlorotic ones of the iron-deficient plants.

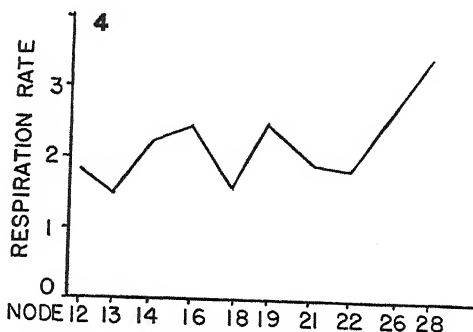
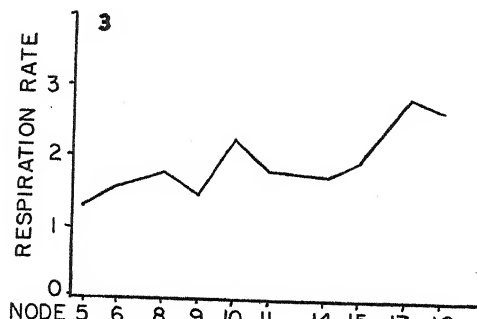
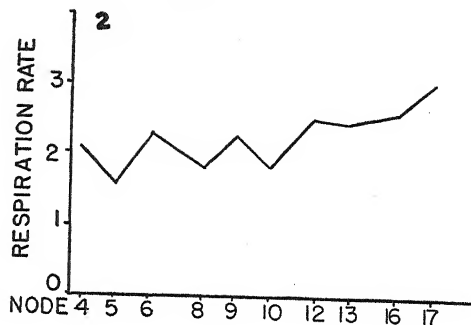
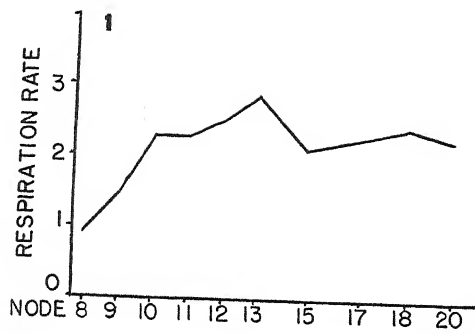
There were no noticeable size differences between the two kinds of plants.

The respiratory gradients of four representative plants of the plus-iron treatment and four of the minus-iron treatment are presented in figures 1-8. The respiration rates (expressed as milligrams of carbon dioxide per gram of dry weight per hour) are plotted along the vertical axes and the numbers of the nodes at which the leaves occurred are plotted along the horizontal axes. The node number was determined by designating the cotyledonary node number 1, and continuing the count from this point to the top of the plant.

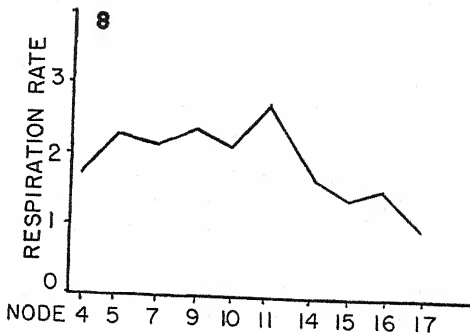
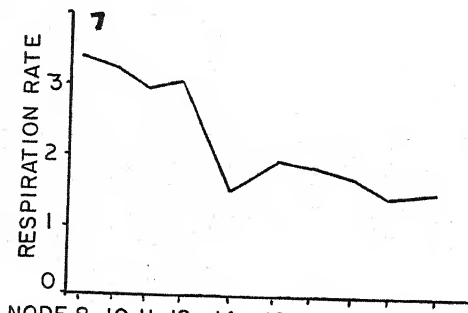
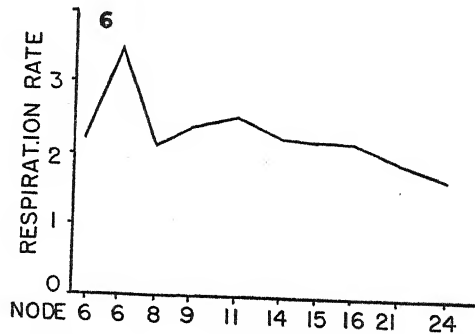
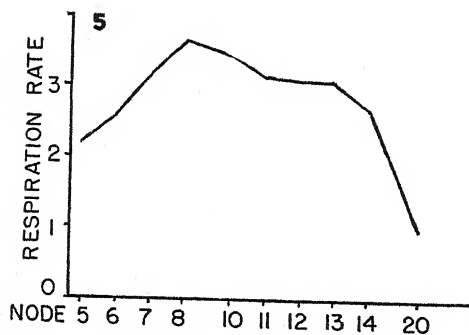
The respiratory gradients for the plants of the plus-iron treatment indicate that the younger leaves were respiring more than the older ones. Such differences are apparently normal, having been reported for other plants (2, 3, 6, 10, 11, 12). The respiratory gradients for the iron-deficient plants indicate that the younger and chlorotic leaves were respiring as much as or less than the older leaves. These gradients are abnormal and indicate a depression of the respiration of the younger chlorotic leaves.

Leaves at adjacent nodes on the same plant may differ greatly in respiration rate. This causes the respiratory gradients to be irregular but does not affect the general trend of the gradients of the two kinds of plants.

Figure 9 is based on the iron gradients of a plant of each treatment, both plants being the same age. These gradients illustrate four findings: (a) the lower older leaves of both kinds of plants contain about ten times as much iron as the younger upper leaves; (b) the gradients of iron content from older to younger leaves are in gradually decreasing order; (c) the leaves of the plant grown with complete nutrient solution contain (with



FIGS. 1-4.—Plus-iron plants: Fig. 1, plant 15, set 1. Fig. 2, plant 6, set 2. Fig. 3, plant 8, set 2. Fig. 4, plant 12, set 2.



FIGS. 5-8.—Iron-deficient plants: Fig. 5, plant 13, set 1. Fig. 6, plant 16, set 1. Fig. 7, plant 18, set 1. Fig. 8, plant 5, set 2.

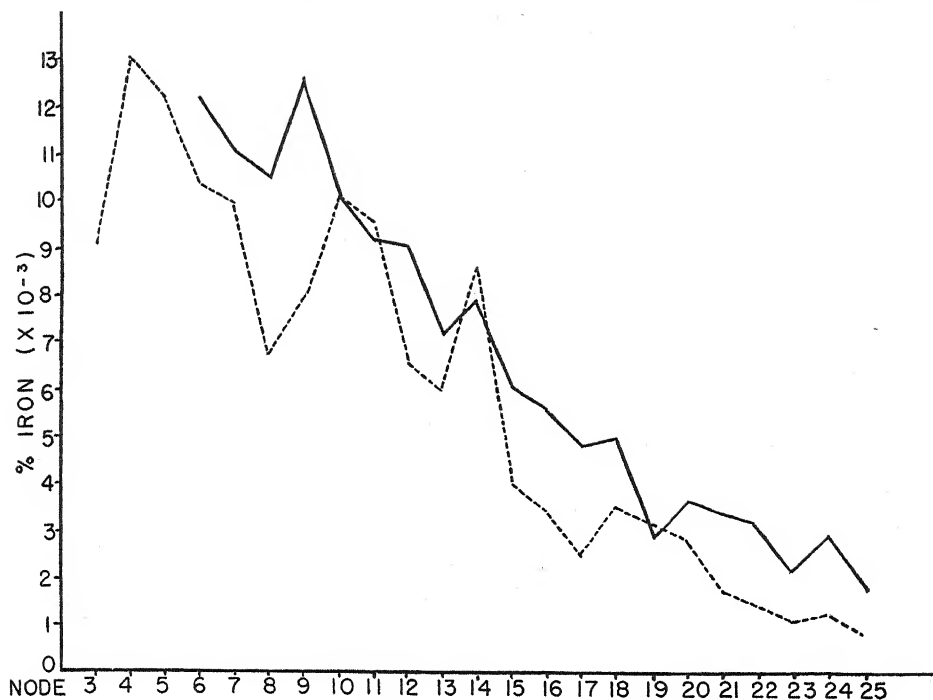


FIG. 9.—Iron gradients in two plants of equal age. Solid line, plus-iron plant; broken line, iron-deficient plant.

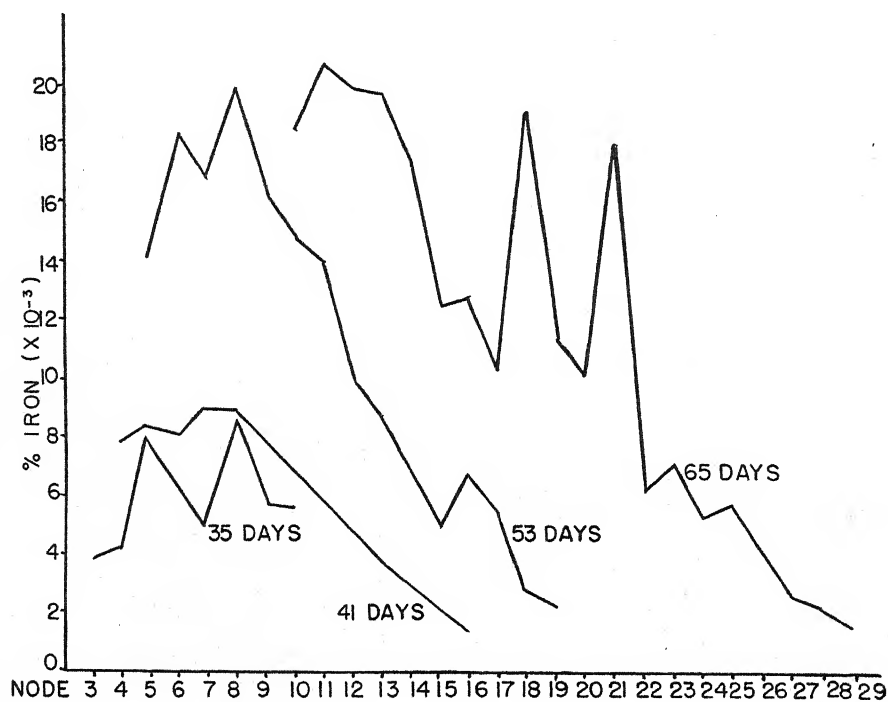


FIG. 10.—Total iron content gradients of plus-iron plants of different ages

a few exceptions) from slightly more than once to more than twice as much iron as the leaves of the iron-deficient plant; (d) this difference is greatest in the younger leaves.

Both the chlorosis and the depression of respiration of the iron-deficient plants

tent, iron content, and respiration rate for a representative plant of each treatment. These data show that the differences in iron supply did not have a great influence on growth of the plants as reflected by these measurements. Direct comparisons of this sort are impossible, because corresponding nodes were not used in every plant and because the plants were of different ages when tested for respiration.

TABLE 2

Node	Green weight (gm.)	H ₂ O (%)	Dry matter (%)	Fe ($\times 10^{-3}$) (%)	Respiration rate
Typical plus-iron plant					
5.....	1.346	84.62	15.38	12.04	1.3
6.....	1.819	86.36	13.64	16.98	1.6
8.....	3.219	87.60	12.40	14.95	1.8
9.....	3.940	87.60	12.40	9.79	1.4
10.....	4.583	86.82	13.18	10.28	2.2
11.....	4.157	87.01	12.99	8.93	1.8
14.....	3.214	85.96	14.04	7.52	1.7
15.....	3.454	85.72	14.28	5.94	1.9
18.....	2.016	85.06	14.94	8.71	2.9
19.....	1.673	84.93	15.07	6.86	2.9
Typical iron-deficient plant					
4.....	1.629	85.87	14.13	11.28	1.8
5.....	1.749	87.53	12.47	11.93	2.3
7.....	4.202	88.83	11.17	8.07	2.2
9.....	4.662	89.44	10.56	10.12	2.6
10.....	4.744	86.81	13.19	8.10	2.4
11.....	4.781	88.72	11.28	7.75	2.8
14.....	5.776	88.22	11.78	4.65	1.7
15.....	4.446	87.69	12.31	3.78	1.4
16.....	3.296	88.53	11.47	2.76	1.4
17.....	2.834	88.14	11.86	3.16	1.0

occur in the leaves with the greatest deficiency in iron—the younger leaves. Figure 10 presents the iron gradients of four plants of similar treatment but of different age. It shows that the iron content of leaves at corresponding nodes is proportional to the age of the plants compared. The older a plant is, the more iron is contained in its leaves.

Table 2 gives the data for green weight, water content, dry-matter con-

The effects resulting from iron deficiency, both chlorosis effects and respiration effects, are localized in the younger leaves. This indicates that iron is not transferred from the older to the younger parts, and that a continuous supply of iron must be available if the younger leaves are to have normal color and normal respiration rate.

The gradients of iron content shown in figure 9 support this interpretation. The gradient for the iron-deficient plant follows the same pattern as that of the plant grown with complete nutrient. If relocation of iron from older to younger leaves had occurred in the iron-deficient plant, its gradient would have been quite different, especially in the region of the older leaves.

It appears to be a general phenomenon that the deficiency symptoms of non-reutilized elements occur primarily in the younger leaves. Chief among such elements are calcium, boron, manganese, copper, and iron. Elements which can undergo reutilization, such as nitrogen, phosphorus, potassium, and magnesium, have deficiency symptoms which occur primarily on the older leaves (5, 7).

In the present work the chlorotic leaves of the iron-deficient plants were found to contain some iron. Lack of it

must have been relative rather than absolute. The problem is therefore one of suggesting a reason for the inactivity of the iron in these leaves. Two mechanisms of inactivation of iron have been suggested by previous investigators.

The first is that the iron is precipitated in the conducting elements because of the high pH of the plant sap. Such precipitated iron would presumably be of no value to the plant. Chlorosis of this type is usually caused by a high calcium content of the soil (2).

The second mechanism of iron inactivation is that involving the iron-manganese ratio. It has been shown by solution culture methods that an iron-deficiency chlorosis will occur if iron is not maintained in the plant in the ratio of 2:1 with manganese (14). The postulated mechanism (15) is briefly as follows. Active iron is ferrous iron, and it tends to be kept in that state by the reducing mechanisms of the tissues. Manganese, because it has a greater oxidation potential than iron, will—when present in excess in relation to iron—tend to keep the iron in the inactive ferric state.

In the present study the first mechanism of inactivation can hardly apply. The concentration of calcium in the nutrient solution was not excessive, and the frequent applications of distilled water which were applied would have kept the calcium from accumulating to an excessive concentration.

The second mechanism of inactivation seems to be the more likely one in the present case. The ratio of iron to manganese in the nutrient solution applied to the iron-deficient plants was far below 2:1. A difficulty with this second mechanism, however, is its premise that active iron is in the ferrous state. While such an assumption may apply to the function of iron in chlorophyll synthesis, it can

hardly apply to its function in respiration. The iron in the cytochrome system functions by way of a cyclic change from the ferric to the ferrous condition. It is the same in the enzyme catalase. Peroxidase occurs and functions only in the ferric condition. It seems more likely that the observed depression of respiration may have been caused by the action of the manganese in preventing or hindering the cyclic valence changes of the iron-containing enzymes in which such changes occur, rather than by the removal of iron from an active ferrous state.

Summary

1. Sunflower plants were grown in sand culture with nutrient solution. One group had iron salt as ferric citrate added to the nutrient solution; the other had no iron salt added.

2. The plants of the minus-iron treatment developed the typical chlorosis symptoms of iron deficiency; the controls were of normal color.

3. Respiration rate was depressed in the young chlorotic leaves of the iron-deficient plants.

4. The chlorotic leaves of the iron-deficient plants contained only half as much iron as comparable leaves of control plants. No relocation of iron from older to younger leaves occurred in the iron-deficient plants. In both the gradients were of decreasing iron content in going from the bottom to the top of the plants.

5. The iron contents of the leaves of a given plant of either treatment were found to be related to the ages of the leaves. The older contained more iron than the younger leaves.

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EMBRYOLOGY OF PASPALUM DILATATUM¹

HUGH W. BENNETT

Introduction

The increasing importance of *Paspalum dilatatum* (Poir.) as a pasture plant warrants more detailed studies of its cytology, morphology, and life history. Information from such studies will have a direct application to the development of improved strains through plant breeding and might reveal the causes of failures of attempted hybridization and the low percentage of seed set. Seed production is the factor limiting the more widespread use of this grass. The object of this study was to trace the developmental morphology of the embryo and to determine not only the manner but also the order and rate of development of the various parts.

The normal development of the embryo would serve as a starting point for investigations of abnormalities in *P. dilatatum* and in other species of the genus.

Previous studies have dealt with determination of the homologies of various portions of the embryo. Relatively little attention has been given to the important stages of development.

According to VAN TIEGHEM (16), the earliest description of the embryo of grasses is that of *Avena* and *Triticum* by MALPIGHI in 1687. He recognized the structures now known as the scutellum, epiblast, and coleoptile. The homologies of these organs are still a controversial subject, as shown by views presented in the more recent papers by AVERY (1), HOWART (4), PERCIVAL (11), and MERRY (9). Detailed views of certain phases of

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embryo morphology have been prepared by VAN TIEGHEM (16), BRUNS (3), KENNEDY (6), and AVERY (2). MCCALL (8) has presented a critical review of the extensive literature and a summary of the various views pertaining to gross morphology and homologies.

Early studies on development were based mainly on observations of whole embryos. NORNER (10) studied the early divisions of the zygote and proembryo of *Hordeum*, *Avena*, *Triticum*, and *Secale* by dissecting out whole embryos and mounting them in glycerin. The oldest stages he figured were just beginning to show organ differentiation. He attempted to classify the arrangement of the cells according to the manner in which they divided.

SOUÈGES (14) traced the parts of the fully developed embryo of *Poa annua* to the tier of cells in the sixteen-celled proembryo. He maintained that the parts of the embryo are determined at least as early as the sixteen-celled stage. He also indicated that there was a definite arrangement of the cells and a regular sequence of cell divisions in the development from the fertilized egg.

RANDOLPH (12) has given a complete description of embryogeny for *Zea*. He found that there was no definite arrangement of the cells and no regular sequence of divisions in the early stages. The sectors of the proembryo in the initial stages of development were so rapidly obscured that it was impossible to determine or predict which sector or sectors gave rise to the root and shoot regions of the plumule-radicle axis. He considered temperature relations as an important factor influencing the rate of development.

MERRY (9) has also given a complete description of the embryogeny of *Hordeum sativum* from the time of fertilization to maturity of the seed. He found no

arrangement of cells or sequence of cell divisions in the proembryo. He shows that the embryos of *Hordeum* varying one day in age have recognizable morphological differences.

LA RUE and AVERY (7) showed a rapid development of the embryo of *Zizania aquatica*, the mature embryo reaching a length of 10 mm. in 19 days. In that species the cotyledon of the embryo extends the entire length of the fruit and often extends around the end of the caryopsis. Growth and development of the *Z. aquatica* embryo are dependent on association with tissues of the plant and it cannot be grown in artificial culture.

Apparently the only reference concerning the embryo of the genus *Paspalum* is that of *P. pubiflorum glabrum* by KENNEDY (6), based on the mature embryo. It is described as having a large radicle; its scutellum-bundle is inserted some distance from the plumule; there is no epiblast; and one foliage leaf is present.

MATERIAL AND METHODS.—Material for embryological studies was obtained from potted plants of strain 56-17 growing in a shaded greenhouse. Samples were collected at specific intervals after pollination. It was necessary to open each spikelet and remove the growing seed or infertile ovule with fine-pointed forceps because of the indurate nature of the lemma and palea. Counts showed that 72% of the ovules were dried and thus not fertile.

The material was killed in Craff and Allen-Bouin (13) fluids. Suction was applied to material younger than 6 days to give total immersion. Material was imbedded in paraffin through an n-butyl-alcohol series. Paraffin sections 10-15 μ in thickness were stained with Delafield's haematoxylin; some sections were stained in safranin fast green.

Observations

Pollen grains germinate very soon after anther dehiscence. The rate of pollen-tube growth within the style has not been determined, owing to difficulty in clearing the feathery, much-branched stigmas. Pollen-tube growth is probably rapid, as the stigmas appear to be dry and dead within 2 hours after anther dehiscence. During periods of rainy weather, with the accompanying high humidity, some stigmas are alive and not wilted the day after anthesis. Fresh pollen on such stigmas has not induced fertilization, however, indicating that this occurs the day of anthesis, possibly very soon after anther dehiscence. Delay in collecting pollen has resulted in a lowered pollen germination on artificial media.

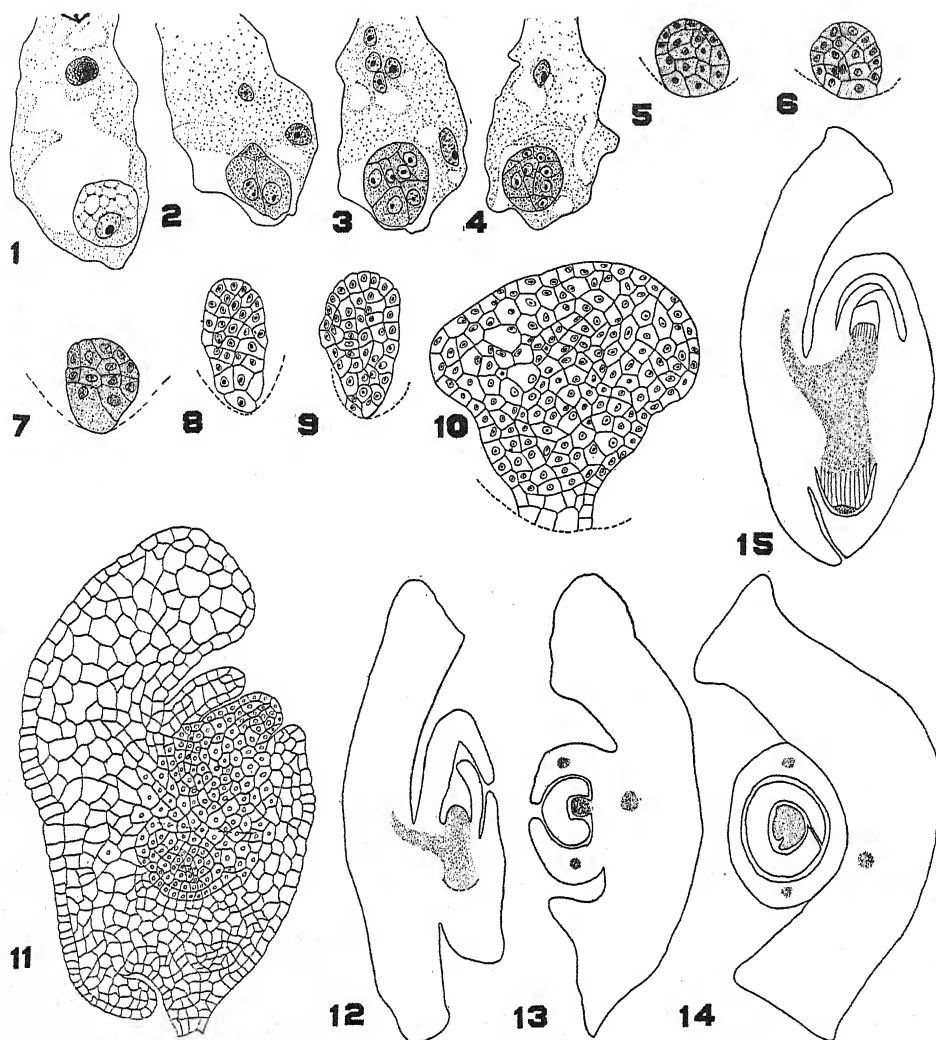
The time interval between pollination and fertilization, under average summer conditions, is between 8 and 12 hours. Zygotes have been found 8 hours after pollination (fig. 1) and small proembryos after 12 hours (figs. 2-4). Twelve hours after pollination the proembryo consists of two to eight cells and is approximately $25-29\ \mu$ in length. The zygote and primary endosperm nucleus undergo division very soon after fusion is completed. The two-celled embryo consists of a small apical cell and a much larger basal cell. There are rapid free nuclear divisions of the endosperm (fig. 3). Twenty-four hours after pollination the proembryo becomes rounded, ranges $33-46\ \mu$ in length, and consists of from eight to sixteen cells (fig. 5). The proembryo begins to lengthen at 36 hours. The apical cells continue to be smaller than the basal cells (fig. 6). The endosperm continues free nuclear.

Differentiation of organs begins during the period from 60 to 72 hours (figs. 7, 8). An increase in the rate of cell division in a posterior subapical region and

in a zone near the apex of the anterior side results in a slight protuberance on each side. This evidently is a preliminary step in the initiation of the coleoptile-radicle axis and of the posterior lobe (left side of fig. 9). The embryo is approximately the same size as 12 hours earlier. Four days after pollination, rapid meristematic activity on the posterior face of the embryo results in enlargement of the posterior lobe, which becomes the basal portion of the scutellum. The suspensor elongates and becomes sharply delimited from the body of the embryo (fig. 10).

Differentiation and growth are extremely rapid during the fourth and fifth days (figs. 10, 11). The coleoptile-radicle axis is $160\ \mu$ long and only slightly oblique to the longitudinal axis of the embryo, which is $148\ \mu$ long. The scutellum enlarges by expansion of the posterior lobe upward and downward. The growing point of the plumule and the coleoptile primordia are very distinct (fig. 11). The coleoptile is formed as a ridge of tissue which nearly surrounds the central growing point. The definite arclike orientation of cells in the lower portion of the axis marks the beginning of differentiation of the coleorhiza. The embryo at this stage is approximately $324\ \mu$ long.

On the sixth day, the ridge of tissue forming the coleoptile has developed more rapidly above than below the central meristem and has formed a sheathing structure that envelops the stem tip. The foliage leaf arises below the stem meristem as a ridge of tissue similar to and opposite that of the coleoptile primordium (fig. 13). Pronounced differentiation of the scutellar trace has taken place. The planes of continued cell division within the lower part of the axis are oriented to produce a dome-shaped zone, which delimits the coleorhiza and pri-



FIGS. 1-15.—Fig. 1, zygote 8 hours after pollination. Figs. 2-4, proembryos 12 hours after pollination. Fig. 5, proembryo 24 hours. Fig. 6, proembryo beginning to lengthen 36 hours after pollination. Fig. 7, proembryo 48 hours. Fig. 8, proembryo 60 hours. Fig. 9, embryo 72 hours. Fig. 10, 4-day embryo; posterior lobe considerably enlarged. Fig. 11, embryo 5 days after pollination, showing distinct coleoptile primordium, plumule growing point, and organization of radicle tip. Fig. 12, embryo 6 days after pollination, showing rapid growth of coleoptile and distinct seedling leaf primordia. Fig. 13, cross-section of 6-day embryo, showing seedling leaf and coleoptile around plumule growing point. Fig. 14, cross-section of 14-day embryo, showing seedling leaf and initiation of another leaf primordium. Fig. 15, mature embryo, 18 days after pollination.

mary root. The scutellum has lengthened to $629\ \mu$ (fig. 12).

The side of the coleoptile next to the scutellum grows faster than that on the anterior side, so that by the seventh day the coleoptile is almost closed, leaving the coleoptile pore on the anterior side. Differentiation of the root cap has begun. The embryo has now reached a length of $684\ \mu$. By the eighth day the leaf primordium has begun to overlap the growing point. The coleorhiza is clearly organized and was differentiated from the lower part of the embryo by the development of the primary root primordium. The scutellar trace is one-half the length of the plumule by the eleventh day. The embryo attains practically mature size in 14 days, having a scutellum length of approximately $780\ \mu$, a plumule-radicle axis of $530\text{--}590\ \mu$, and a thickness of approximately $250\ \mu$ (fig. 14).

The seedling leaf continues to lengthen and is curved around the growing point at 14 days. Another leaf primordium has begun to differentiate as a small protuberance above the stem meristem directly opposite the first leaf. This second leaf fails to develop further, and there is only one seedling leaf in the mature embryo (fig. 15).

Discussion

Following division of the zygote, the subsequent planes of cell division in the proembryo are irregular, and apparently no special significance can be attached to the sequence of cell division or to the arrangement of cells in the early development of the embryo. This is in agreement with the findings of RANDOLPH (12) with corn and of MERRY (9) with barley and indicates that the factors controlling growth of the proembryo for the first 60 hours affect the embryo as a whole, rather than definite cells. SOUÈGES (14) held that

parts of the embryo are already determined in particular cells in the sixteen-cell embryo. NORNER (10) also attempted to classify the arrangement of cells according to the manner in which they divided.

The proembryo is almost round for the first 60 hours after pollination and is comparable to the same stages of growth in *Eragrostis cilianensis* (All.) Link., as described by STOVER (15). During these stages, the only perceptible differentiation is the difference in the size of the apical and basal cells. During this period, growth of the proembryo is limited chiefly to the apical region and may suggest a gradient from the apex to the base of some factors which may control the rate of division of the cells. The appearance of smaller cells in certain particularly highly meristematic parts of the embryo at 3 and 4 days may indicate localization of similar factors in those regions.

The development of the embryo from initiation of the axis (3 days) to maturity follows that of the ordinary grass type of embryo as described by LA RUE and AVERY (7), RANDOLPH (12), MERRY (9), STOVER (15), and summarized by MCCALL (8).

The mature embryo of *P. dilatatum* differs from the embryo of many grasses in that it has only one foliage leaf and no epiblast. It differs from the embryo of *P. pubiflorum glabrum* (6) in that the axis is more oblique to the main body of the embryo.

The embryo of *P. dilatatum* is mature 14–18 days after pollination. This is relatively rapid in comparison with development of the embryo in maize, which requires 45 days (12), and barley, which requires 35 days (9). The rate of maturity is slightly faster than that found for *Zizania aquatica* by LA RUE and AVERY (7), who regarded the 19-day-old

embryo to be mature but the seed "un-ripe." The fastest rate of development of a grass embryo is that reported by JENKINS (5) for *Poa annua*, in which 82% of the caryopses were mature 14 days after pollination.

Some evidence of cleavage polyembryony has been found in *P. dilatatum*, which might well be one cause of the low yield of viable seed.

Summary

1. The time interval between pollination and fertilization of *P. dilatatum*, under average summer conditions, is 8-12 hours.

2. The endosperm nuclei are free for the first 2 days. There is no consistent

arrangement or zonation of cells in the young embryo.

3. Initiation of the coleoptile-radicle axis begins in 60-72 hours.

4. Development of the embryo is extremely rapid from the fourth to the eighth day and is structurally complete in 14-18 days.

5. The mature embryo has one foliage leaf and a distinct radicle with a root cap.

6. There is no epiblast.

The writer wishes to express his appreciation to Dr. J. E. SASS for his valuable suggestions during the study of this phase of the problem.

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GROWTH AND DEVELOPMENT IN RANGE GRASSES. IV. PHOTO-
PERIODIC RESPONSES IN TWELVE GEOGRAPHIC
STRAINS OF SIDE-OATS GRAMA¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 562

CHARLES E. OLMSTED

Introduction

Many recent and some older investigations have emphasized the characteristic growth and habits of strains of native species obtained from different locations in their respective distributional areas (often involving altitudinal or latitudinal gradients) when they are grown side by side under nursery or greenhouse conditions (3, 7, 13, 18, 19, 25, 26, 27, 29, 32, 33, 34). Irrespective of the original causes of the genotypic differentiation which has brought about such diversity, it has usually resulted in a high degree of physiological and often of morphological adaptation to the environment in which a strain (ecotype) is now native, as compared with its responses in the native environments of related strains (7, 29, 34). Such differentiation and adaptation have long been known for agronomic and horticultural crops and some forest trees and are the basis for much of the work in plant breeding, variety testing, studies of acclimatization, etc.

Much interest has been shown in the differing photoperiodic responses of various strains and varieties of many species of cultivated plants (2, 4, 5, 8, 9, 10, 11, 14, 20, 24, 36). The range of phenotypic expression induced by different photoperiods is of obvious applied significance in the seasonal and latitudinal adaptation of any particular strain. Possible variation in photoperiodic responses among strains of native species has been largely ignored, or has been studied only

in a limited number of strains, often not systematically selected (1, 2, 14, 15, 30, 31). Consequently, it seemed that an investigation of the photoperiodic responses of different latitudinal strains of a widely ranging native species (with consequent differences in range of natural photoperiods in the different localities) might yield data of considerable interest.

Earlier work (23) had demonstrated that a strain of side-oats grama grass (*Bouteloua curtipendula* (Michx.) Torr.) from southern Arizona consisted largely of short-day plants with a critical photoperiod between 14 and 14½ hours. Since northern strains flower on much longer natural photoperiods, it was thought that this species might exhibit considerable differentiation in photoperiodic responses or requirements. Strains from different latitudes, grown together in a number of places (13, 16, 19, 26, 27), have shown morphological, physiological, and cytological diversity, correlated in part with latitude of origin.

Side-oats grama occurs over much of eastern, central, and southwestern United States, extending into Canada on the north (southern Saskatchewan), southward into Mexico, and in South America to Argentina (16, 17). In the United States it is of greatest importance in the western part of the prairie region and at certain altitudes in the Southwest, where it is a valuable forage plant, for both hay and grazing. It has shown considerable promise in the artificial or natural reseeding of drought-stricken, overgrazed, and eroded land, and it is thought by some to be the most promis-

¹ This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

ing grama for domestication (16, 18, 27, 35). A knowledge of the photoperiodic responses of some of its strains should be of value in: (a) helping to explain the observed differences among them when grown together under nursery conditions (13, 16, 19, 26, 27); (b) interpreting the factors affecting their vegetative and reproductive habits during the growing season; (c) assisting practical plant breeders to select or breed strains for re-seeding purposes by indicating the possible restrictions that photoperiodic sensitivity would impose. The investigation might also be of interest from the standpoint of the evolution of adaptive physiological responses in general and of the operation of natural selection as the range of a species is extended (7, 22, 33). The latter has implications for plant geography, especially with respect to environmental factors limiting migration and possible changes in the limiting values of factors for particular species in the course of time.

Experiments were initiated in the spring of 1942 at the University of Chicago and have been continued since. The present paper is a survey of the photoperiodic responses of twelve strains from different latitudes during 1942 and 1943. Additional work on some of these strains is in progress. Related work by LAVIN (21) on geographical strains of blue grama (*Bouteloua gracilis* (H.B. K.) Lag.) has not yet been published.

Material, methods, and environmental conditions

Seed of twelve strains of side-oats grama (fig. 1) was obtained from various sources (table 1). Strains were designated by number as indicated. The latitudinal range from San Antonio, Texas, to Cannonball, North Dakota, is approximately 17°. The periods between

sunrise and sunset at these two stations at the summer solstice are approximately 14 and 16 hours, respectively (effective photoperiods probably near 15 and 17 hours, allowing for dawn and twilight). The rate of increase to or decrease from these maxima to or from the nearly equal period of slightly over 12 hours from sunrise to sunset at both stations at the equinoxes (effective pho-

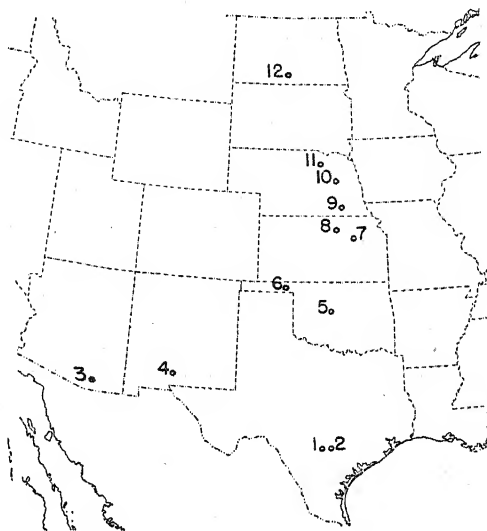


FIG. 1.—Geographical sources of strains numbered as shown.

toperiod under 13 hours) is obviously greater at the northern station.

In the 1942 experiment, 225 8-inch unglazed clay pots were evenly filled and packed with a silt loam soil of prairie origin (near Chicago) and of medium fertility. They were divided into five equal series (45 pots in each). On April 11, uniform caryopses were planted 1 cm. deep at evenly spaced intervals at the rate of 30-45 per pot, as governed by previous germination tests. Thirty-five pots in each series were planted with strains nos. 1, 4, 5, 7, 9, 11, and 12 (five pots per strain) and ten pots in each

series with strains nos. 2, 3, 6, 8, and 10 (two pots per strain).

Immediately after the prompt and uniform germination (all seedlings to emerge appeared by April 15), the five series were placed on photoperiods of 9, 13, 16, and 20 hours, and natural day-

out the season. The fourth and fifth series were placed in separate greenhouse rooms. The latter was on natural day (N series) and the fourth received artificial illumination after sunset to provide a 20-hour photoperiod. The intensity of the supplementary light, from 200-

TABLE 1
SOURCE OF SEED OF TWELVE STRAINS OF SIDE-OATS GRAMA

Strain no.	Point of origin of strain	Approximate latitude	Harvested from	Year harvested	Received from	Accession no. of agency
12.....	Cannonball, North Dakota	46½	SCS Nursery, Mandan, North Dakota	1941	R. W. Carpenter, SCS, Mandan, North Dakota	NDG204-41
11.....	Holt County, Nebraska	42½	SCS Nursery, North Platte, Nebraska	1941	E. C. Conard, SCS, Lincoln, Nebraska	373-41
10.....	Platte County, Nebraska	41½	SCS Nursery, North Platte, Nebraska	1941	E. C. Conard, SCS, Lincoln, Nebraska	2409-41
9.....	Jefferson County, Nebraska	40	Field	?	J. E. Weaver, Univ. of Nebraska, Lincoln, Nebraska
8.....	Cloud County, Kansas	39½	Field	1940	E. C. Conard, SCS, Lincoln, Nebraska	3177-40
7.....	Alma, Kansas	39	Field on farm of August Feyh	?	Donald R. Cornelius, SCS, Manhattan, Kansas	KG1823
6.....	Beaver County, Oklahoma	37	Field	1941?	D. A. Savage, BPI, Woodward, Oklahoma
5.....	El Reno, Oklahoma	35½	SCS Nursery, Manhattan, Kansas	?	Donald R. Cornelius, SCS, Manhattan, Kansas	KG1234
4.....	Las Cruces, New Mexico	32½	Field	1941	Anton Berkman, Texas Coll. of Mines, El Paso, from U.S. Forest Service
3.....	Santa Rita Exp. Range, Arizona	32	Field	1938	R. A. Darrow, Univ. of Arizona, from U.S. Forest Service
2.....	San Antonio, Texas	29½	SCS Nursery, San Antonio, Texas	1941	D. E. Griffith, SCS, San Antonio, Texas	T-3846 (1-41)
1.....	Bexar County, Texas	29½	SCS Nursery, San Antonio, Texas	1941	D. E. Griffith, SCS, San Antonio, Texas	T-3841 (1-41)

length. Two series on two movable trucks received natural daylight for 9 hours between 8:00 and 5:00 P.M. (CDT) but were rolled into ventilated light-proof sheds for the remainder of the 24 hours. One of these two series was given 4 hours of artificial light from 5:00 to 9:00 P.M., to provide a 13-hour photoperiod. The third series on a stationary truck in the same room received supplementary illumination after sunset to furnish a 16-hour photoperiod through-

watt Mazda lamps mounted in individual reflectors over the different series, varied from 100 to 180 foot-candles (Weston sunlight meter) at average foliage height, depending upon pot position and plant height.

Responses on Chicago natural day-length may be usefully compared with behavior under field conditions. A 13-hour photoperiod is near or below the lower limit of the photoperiodic range on which any of the strains flower under

natural conditions, while 16 hours of light per day is somewhat below the maximum to which the northern strain is naturally subjected during part of its growth period. Light periods of 9 and 20 hours were used to determine whether critical photoperiods occur above or below the other values. It should be remembered that plants in the 9-, 13-, 16-, and 20-hour series were subjected to daily photoperiods of constant length throughout the season, while in the N series they were under the influence of the changing natural photoperiods. The maximum natural photoperiod at Chicago at the solstice is 15 hours and 14 minutes (sunrise to sunset).

Plants were thinned to ten per pot during the first 2 months of growth. During the summer, efforts were made to maintain similar favorable moisture, temperature, and natural light intensity values among the various series. Practically all plants were harvested the fourth week in September by clipping the tops at approximately 2.5 cm. above the soil surface. One pot of each strain under each treatment was then washed out for examination of roots and rhizomes. The remaining pots were placed on natural photoperiod during the winter of 1942-43, except as indicated in succeeding paragraphs, and left in the same greenhouses as during the summer. During October vegetative growth was resumed, the three southernmost strains (1-3) soon reaching average foliage heights of 25-30 cm. on the shorter days of autumn, while growth of the more northern strains was proportionately less with increase in latitude of origin, with a tendency to develop a rosette habit, irrespective of previous treatment. There was little internodal elongation in any strain at this time, and no

further flowering occurred until May and June of 1943.

In November the temperature was reduced in the house containing the series previously on 20-hour photoperiod, and values ranging from 35° to 55° F. were maintained throughout the winter and early spring. In late April, 1943, they again approached those of 60°-75° which had been maintained in the other two houses over winter. Plants in the cool greenhouse were practically dormant from November to May. The foliage which had been produced in October died back in November and December to within 2.5 cm. of the soil level. These plants resumed growth slowly about May 1, and by June 27 all strains had mostly attained average foliage heights of approximately 30 cm., although no flowering and little internodal elongation occurred. Some plants of strain no. 1 not clipped in September resumed growth at the tips of the stems formed in the summer of 1942. This 20-hour series of 1942 was left on natural photoperiod during the summer of 1943 and was designated 20-N.

The N series of 1942 was left in a warm greenhouse over winter, and because of bench position it received natural light of lower intensity than did the other four series during the winter months. The plants also became dormant in November and December, most of them dying back to the ground in a manner comparable with those which had been on 20-hour photoperiod in the summer. They resumed growth slowly, the southern strains first, in early April. By May 25 the three southern strains had average foliage heights of about 30 cm., while the northern strains were still rosettes. No culm elongation had occurred by this date, when this series (N-N) was placed with the 20-N series.

Plants which had been on 9-, 13-, and 16-hour photoperiods until late September, 1942, were left in a warm greenhouse with the maximum natural light intensity possible in the greenhouses at Chicago in the winter months. They also mostly became dormant in November and December, but not quite so markedly as the other two series. Most of the foliage produced in October by the three southern strains remained green. Their further growth was limited after December until about March 15, when growth was resumed—both from new tillers and from those of the preceding autumn. By May 21 these three southern strains averaged about 40 cm. tall, although there had been little culm elongation. The apparent depth of dormancy in the more northern strains over winter increased with latitude of origin. In the North Dakota strain (12) practically all foliage died, so that the only visible green tissue in December was located in stem bases or small tillers in the crown within 2 inches of the soil level. Some plants of this strain were then given supplementary illumination (values comparable with those given above) to provide a 20-hour photoperiod during January, 1943. Growth was resumed, and within 30 days the average foliage height was about 25 cm. No other plants of any strain on natural photoperiod grew at this time. This new growth died back to the ground when the pots were placed on 8 hours of natural light per day in February. The other plants of the nine northern strains in these three series resumed growth during the latter part of March and early April, height growth being more rapid in the southern strains, so that for about a month there was a decreasing gradient in height from southern to northern strains. By mid-May size differences

were less pronounced, all plants ranging 30-45 cm. tall. There was some culm elongation in the northern strains, and a few plants of strains 10 and 12 showed exerted inflorescences on May 15. These apparently had developed in response to the increasing length of the natural photoperiod.

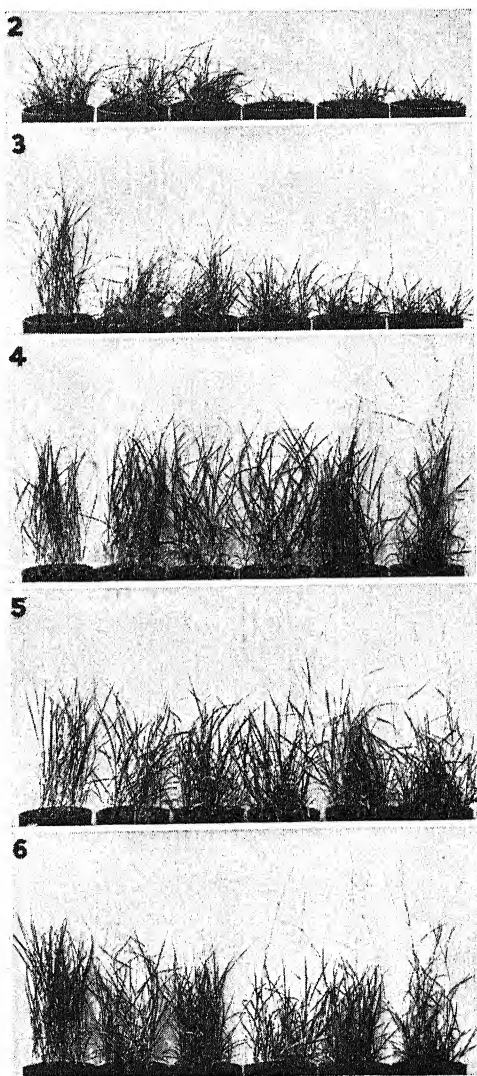
On May 21 all plants of the 9-, 13-, and 16-hour series of 1942 were clipped to within 3 cm. of the soil level. These pots were then redistributed among four series on 9-, 13-, 16-hour, and natural photoperiods during the summer of 1943. One pot each of strains 1, 4, 5, 7, 9, 11, and 12 from each of the 9-, 13-, and 16-hour series of 1942 was available for each of the four series of 1943, so that a total of thirty plants of each of these strains was placed on each new treatment. The one pot available for strains 2, 3, 6, 8, and 10 from each of the 9-, 13-, and 16-hour series of 1942 was placed on a similar photoperiod in 1943, but none were available for the N series. In 1943, because of "blackout" regulations, all plants received supplementary illumination in light-proof sheds. Thirteen- and 16-hour photoperiods consisted of 9 hours of natural daylight with 4 and 7 hours, respectively, of supplementary illumination varying in intensity from 100 to 180 foot-candles.

Six major series were thus run in the summer of 1943. One series (N-N) consisted of forty plants each of strains 1, 4, 5, 7, 9, 11, and 12, and ten plants each of the other five strains which had grown on natural photoperiod in 1942, had been clipped in September, and were then left on natural photoperiod for the ensuing months in a warm greenhouse. The second series (20-N) consisted of the above number of plants of each strain, grown on 20-hour photoperiod in 1942 before clipping in September, on

natural photoperiod after clipping, and subjected to cool conditions during the winter of 1942-43. These two series were placed together on May 25, 1943, and both were allowed to grow on natural photoperiod thereafter. The other four series were as outlined in the preceding paragraph, with thirty plants each of strains 1, 4, 5, 7, 9, 11, and 12 on each of 9-, 13-, 16-hour, and natural photoperiods; and ten plants each of strains 2, 3, 6, 8, and 10 on each of the 9-, 13-, and 16-hour photoperiods. In these four series all plants had been clipped in September, 1942, and were again clipped on May 21, 1943. After this date an effort was made to maintain similar environments for all six series, except for duration of the light periods. Development and sequence of flowering were followed until late October, 1943.

Supplementary illumination was continued in 1943, after the conclusion of the major experiments, to provide some plants with a 16-hour photoperiod during the ensuing winter months, while others were placed on natural photoperiod. All were in the same warm greenhouse after November 1. Some foliage of plants of all strains remained green when provided with supplementary light, although new growth was limited after December and no culm elongation or flowering occurred in any strains during the winter months. In those plants not receiving supplementary illumination after October, dormancy developed comparable with that of the preceding year. Some of the foliage of the southern strains remained green but without new growth, while foliage of the northern strains died back. In late December all plants were supplied supplementary illumination to provide a 16-hour photoperiod. They grew slowly in January, and by mid-February new foliage had

developed on all strains to lengths of 15-20 cm., but no flowering had occurred by late March. ROGLER (26), from experiments in North Dakota and Minnesota, reported that strains of this species



FIGS. 2-6.—Ten plants of side-oats grama per pot on August 6, 1942, 118 days after germination. Left to right: strains 1, 4, 5, 9, 11, 12, in latitudinal sequence from south to north; grown on photoperiods of: fig. 2, 9 hours; fig. 3, 13 hours; fig. 4, 16 hours; fig. 5, 20 hours; fig. 6, Chicago natural day-length.

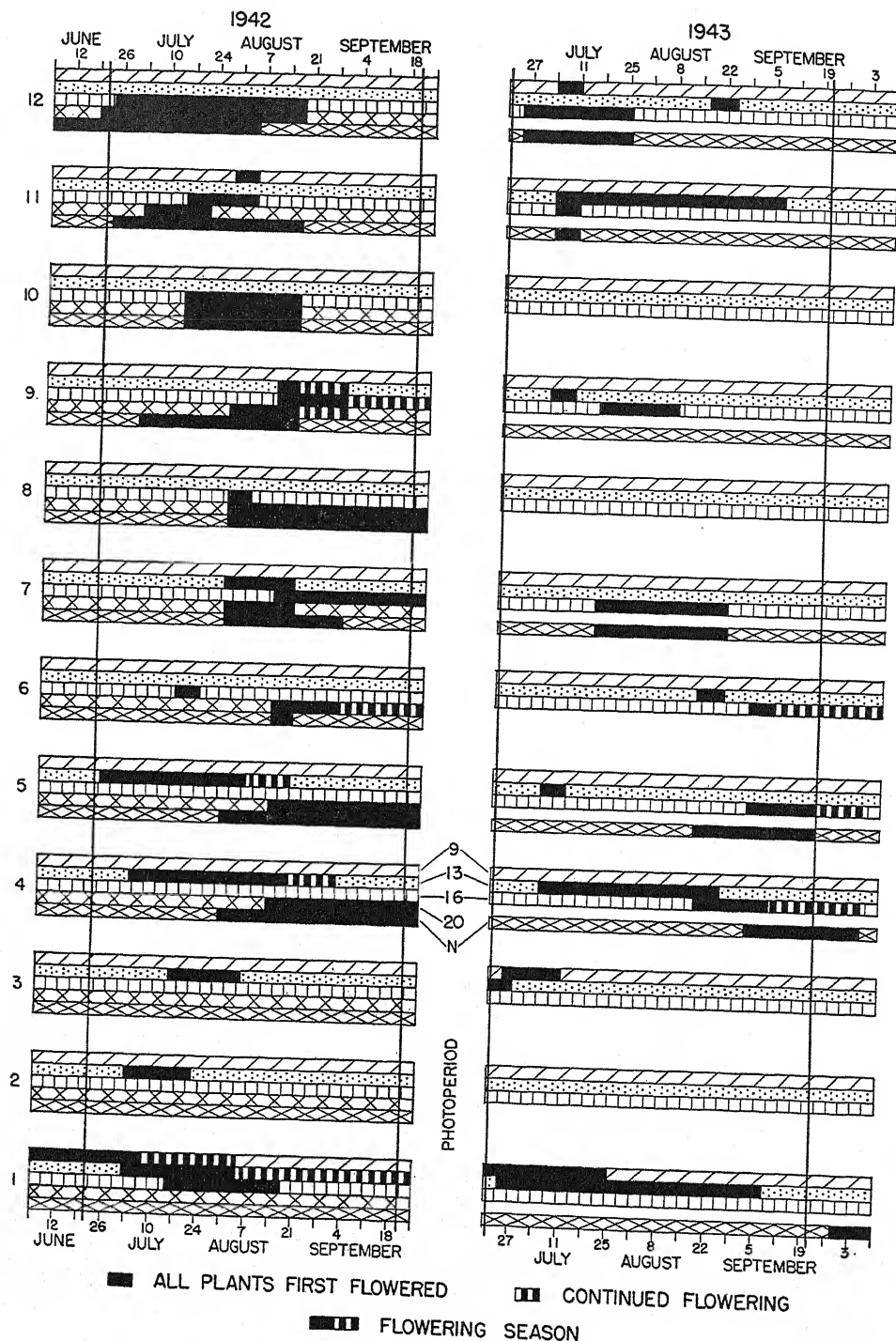


FIG. 7.—Flowering seasons of twelve strains of side-oats grama in 1942 and 1943 grown on photoperiods (hours) as indicated. Germination on April 11, 1942; plants clipped May 21, 1943. Solid bars show period in which individual plants first exerted inflorescences; broken bars show period of continued flowering by one or more plants.

from a wide latitudinal range tended to become dormant in the fall, even at high temperatures, but he did not suggest any explanation.

Because of failure to flower and limited growth in the greenhouses during winter at Chicago, critical experiments on photoperiodic responses of this species are possible here only during late spring and summer. Conclusions must therefore be based chiefly on the responses observed during the first and second summers of growth. Development during the second summer was apparently affected to some degree by over-wintering conditions and whether the plants were clipped previously during May (figs. 7, 8; table 3). Those plants kept in a warm greenhouse under the highest possible natural light intensity during the winter months of 1942-43 showed less profound dormancy than did the others. They renewed growth earlier in the spring and grew more vigorously before they were clipped on May 21 than did those on other winter treatments. Winter chilling is apparently unnecessary for vigorous growth the following year, while the appearance of inflorescences on two of the northern strains by mid-May without winter chilling suggests that cold temperatures are not needed for the initiation of reproductive activity. SHEPHERD (28), however, reported that a strain from Hays, Kansas, grew more vigorously there in the winter months from sods brought into the greenhouse on December 30 than from green sods brought in on October 13 which had become "dormant and sere" by November 18, although watered. The latter sods renewed growth in 21-69 days after treatment with potassium thiocyanate on November 18, and in 54-69 days without chemical treatment on natural and 16-hour photoperiods, respectively. None of

SHEPHERD's plants flowered during the winter months. His plants on 16-hour photoperiod grew more vigorously than those on natural daylength. No freezing or chemical treatments to break dor-

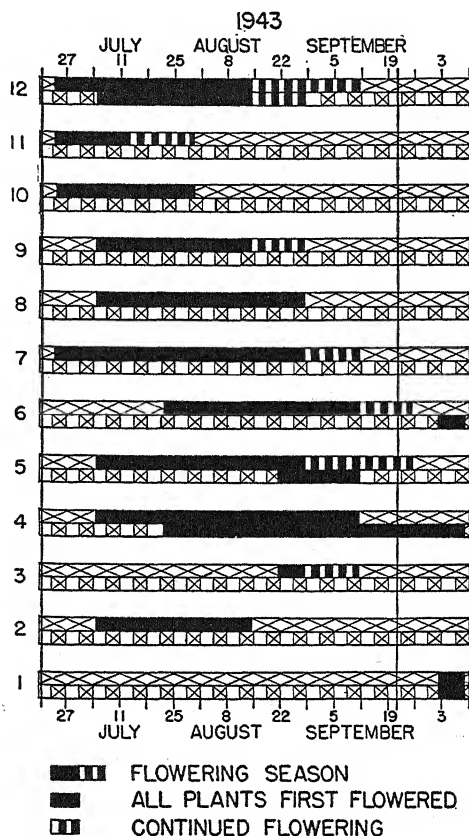


FIG. 8.—Flowering seasons of twelve strains of side-oats grama in 1943 on Chicago natural photoperiod. Germination on April 11, 1942; plants not clipped in 1943. Upper and lower of each pair of bars are N-N and 20-N series, respectively.

mancy in the winter months have been tried by the writer, but the results with supplemental illumination in autumn and winter months suggest that dormancy of these strains in warm greenhouses may in part be induced by a complex of external conditions, among which decreasing photoperiod and decreasing light intensity are important. Under

field conditions in their native environments, cool temperatures and drought are probably equally if not more important, although undoubtedly decreasing photoperiod has an effect. ROGLER (26) at Mandan, North Dakota, and HOPKINS (19) at Hays, Kansas, working with the same species, have both reported that in nursery-grown plants from a wide geographical range there was progressive delay in maturity—and subsequent dormancy—with decrease in latitude of strain origin. The southern strains were regularly injured by frost at these stations before they were dormant, while the strains from latitudes near or north of these stations became semidormant several weeks before the first frost. The results reported in this paper suggest that these responses may have been due largely to the differing effects of the decreasing photoperiods on the various strains used by these investigators.

There is certainly need for more investigation on the factors which induce and break dormancy and which prevent flowering in this species in the greenhouse in the winter months. Enough has been done, however, to suggest that winter dormancy or lack of it probably is of little importance in affecting photoperiodic responses in the summer; if the plants resume growth early in the spring—as they did in all except the 20-N series. The difference in flowering seasons and vigor of flowering between the N-N and 20-N series in 1943 (fig. 8; table 3) can be attributed in part to the delay in renewal of growth of the latter, owing to its subjection to cool temperatures in April, as well as to the different treatments over winter.

The photoperiodic treatments of 1942 seemed to have relatively little effect on responses the following year. The re-

sponses were similar in the three pots of each of the seven strains in the 9-, 13-, 16-hour, and N series of 1943 which had been grown on three separate treatments in 1942. It seemed justifiable, therefore, to average the 1943 data within each group of three pots. These results are in contrast with TINKER's (31) observations on several species of grasses. He reported considerable effect of different photoperiodic treatments one season upon growth and flowering the following year.

The four series placed on 9-, 13-, 16-hour, and N photoperiods on May 21, 1943, were clipped on that date in order to remove any developing inflorescences and possibly to prevent aftereffects which could be attributed to photoperiodic induction (6) of some of the strains during their exposure to the increasing natural photoperiods of 12 or more hours when they resumed growth in March and April. A lack of much aftereffect of previous photoperiod when plants were so clipped at the time of reciprocal transfers among different photoperiods has been reported (23) for strain 3. The May clipping may not have been effective in removing all inflorescence primordia. Two plants of strain 12 showing several emergent inflorescences on May 21 had each again produced one visible but weak inflorescence 44 days later (on July 11) on the 9-hour photoperiod (fig. 7; table 2). Since these were the only plants of the nine northern strains flowering in the 9-hour series in 1943, these inflorescences may already have been initiated before the plants were placed on this photoperiod and escaped clipping owing to proximity to the soil level. It has not yet been possible to work out the developmental morphology of all the strains or the minimum length of time required from initiation to exsertion

of inflorescence (12). It is known that, after initiation, flower buds in certain chrysanthemums may persist on photoperiods unfavorable originally to initiation. Their further development may be retarded, or completely inhibited, until the proper photoperiod is provided (2). In other species, primordia may be initiated under a wide range of photoperiods but will develop into visible flowers only over a more limited range (5, 30). In the Arizona strain of side-oats grama, inflorescences may be initiated and exerted in less than 4 weeks of growth after clipping when on appropriate photoperiod (23). They may also be rapidly initiated on elongated vegetative culms. Whether this is true of the other strains is not known, although exerted inflorescences appeared at 30 and 34 days after clipping on strains 1 and 12 on 9- and 16-hour photoperiods, respectively (table 2).

Clipping in May probably caused some reduction in the vigor of growth and flowering of plants so treated. This is apparent to a limited extent in comparing the 1943 N series so clipped with the unclipped N-N series (figs. 7, 8; table 3). The length of the flowering season of certain strains in the former series was probably shortened also, since the clipping undoubtedly destroyed some inflorescences which otherwise would have been exerted in late May or June. This should be borne in mind in comparing the three N series of 1943 (N, N-N, 20-N) which had not received identical treatment at any time until late May, 1943.

It was not possible to use as many or as large pots for these experiments as for those previously reported (23) or to transplant as originally planned. Consequently, there was considerable competition among the ten individuals in

each pot, and growth and flowering were restricted, especially in the second year, although the conditions were no more severe than the species encounters in nature. Plants of the same strain and treatment were not so uniform in size as in previous experiments, and undoubtedly some individuals failed to flower which would have done so if less crowded. While the quantitative data are therefore less decisive, and the variation among individuals in each strain on each treatment cannot be ascribed chiefly to genetic differences within the populations represented, yet the qualitative differences—both vegetative and flowering—among strains and treatments seem sufficiently clear-cut to point to definite conclusions as to the nature of the photoperiodic responses of the various strains.

Results

TIME REQUIRED FOR FIRST FLOWERING

The beginning and progress of flowering were measured by the exertion of inflorescences from the surrounding leaf sheaths. Time and materials did not permit dissection methods, which would have shown initiation of inflorescences in the apical meristems (12). For the purposes of this investigation, however, the effects of different photoperiods in inducing, allowing, or preventing development of fertile inflorescences are of equal significance with their effects on the initiation of floral primordia.

FLOWERING OF YOUNG PLANTS, 1942.—Table 2 shows the approximate number of days after germination when the first inflorescence appeared in each strain on each treatment. It was formed on the primary axis except in three cases. While these data are based on only one plant

from each population, they are indicative of the responses of the various strains. If the columns are read both vertically and horizontally, the failure

TABLE 2

NUMBER OF DAYS UNTIL FIRST FLOWERING,* AFTER GERMINATION ON APRIL 11, 1942, OR AFTER CLIPPING ON MAY 21, 1943, FOR STRAINS AND PHOTOPERIODS (IN HOURS) AS INDICATED

STRAIN NO.	PHOTOPERIODS (1942)					PHOTOPERIODS (1943)			
	9	13	16	20	N	9	13	16	N
12.....			73	60	55	44†	88‡	34	34
11.....	109†		95	81	73		44	44	44
10.....			95	95	95				X§
9.....		123†	123	109	81		44	59	
8.....			109	109	109				X§
7.....	109†		123	109	109			59	
6.....			95	123	123		88	103	X§
5.....		73		123	109		44	103	88
4.....		81		123	109		44	88	103
3.....		95				34	31		X§
2.....		81							X§
1.....	55	81	95			30	34	193	130

* Measured by exertion of inflorescence from surrounding leaf sheath.

† In 1942 data, where so indicated, inflorescences appeared only on tillers; in all other 1942 data they were exerted first on primary axes.

‡ Inflorescences weak and sterile; primordia possibly initiated before clipping, since plants were flowering when clipped.

§ No plants of this strain on this photoperiod.

or progressive delay in onset of flowering among the various strains in one treatment thus shown, or of the several treatments of one strain, suggests strongly that the southern strains 1, 2, and 3 are either short-day or intermediate (2) in their photoperiodic responses, while the northern strains are long-day. Data for plants on Chicago natural photoperiod indicate, as do many other data, that the three southern strains respond in a different manner from the nine northern ones. Although there was a more or less progressive increase in the delay of first flowering of the nine northern strains with decrease in latitude of origin, some plants of all nine strains apparently are able to initiate and mature inflorescences on the long natural photoperiods of June and July at Chicago. Indeed, some plants of all nine strains did so on a 20-hour photoperiod, although with increasing delay with decrease in latitude of origin. The three southern strains, however, had failed to produce inflorescences when clipped on September 23, on either Chi-

TABLE 3

PERCENTAGES OF PLANTS FLOWERING* BY SEPTEMBER 23, 1942 AND BY OCTOBER 19, 1943

STRAIN NO.	GERMINATION APRIL 11, 1942 PHOTOPERIODS (1942)					CLIPPED MAY 21, 1943 PHOTOPERIODS (1943)				NOT CLIPPED 1943	
	9	13	16	20	N	9	13	16	N	N-N	20-N
12.....	0	0	60	52	78	7†	3†	30	13	58	15
11.....	2	0	16	12	40	0	10	3	3	10	0
10.....	0	0	15	25	50	0	0	0	X†	50	0
9.....	0	4	8	14	22	0	0	13	0	23	0
8.....	0	0	5	10	20	0	0	0	X†	70	0
7.....	0	4	12	6	8	0	0	10	7	28	0
6.....	0	0	5	15	5	0	10	10	X†	20	10
5.....	0	8	0	6	12	0	7	20	10	13	5
4.....	0	10	0	12	10	0	7	17	10	10	15
3.....	0	15	0	0	0	20	10	0	X†	10†	0
2.....	0	35	0	0	0	0	0	0	X†	40†	0
1.....	66	96	2	0	0	67	93	0§	13	13	3

* One or more inflorescences per plant exerted above surrounding leaf sheaths.

† Inflorescences weak and relatively sterile.

‡ No plants of this strain on this photoperiod.

§ Seven % on December 1, 1943.

cago natural or 20-hour photoperiods, or in the 16-hour series—except for one plant of strain 1. There is apparently a critical photoperiod above which plants of these three strains will not flower.

FLOWERING OF SECOND-YEAR PLANTS AFTER CLIPPING, 1943.—Although the data for these series (table 2, 1943) are not based on as many plants as in the 1942 series, and some strains failed to flower on any treatment, in general the same conclusions may be derived. The practical failure of flowering of the nine northern strains on the 9-hour photoperiod, and the failure or great delay of the three southern strains in the 16-hour and N series, again suggest a fundamental genetic difference between the two groups of strains. The northern strains showed an increased delay in flowering on 16-hour and N photoperiods with decrease in latitude of origin.

FLOWERING SEASONS

The previous discussion is based primarily on the length of time required for the first plant to flower in each strain on each treatment. More significant conclusions are based on the sequence and duration of flowering of all plants. From data on the percentages of plants of each population which either had flowered or were in flower, and from the numbers of inflorescences which had appeared on individual plants at various times throughout both summers, graphs and a table were constructed (figs. 7, 8; table 4) depicting the flowering season of each strain on each treatment. These show the periods during which additional individual plants of each population first flowered (solid bars) and the continuing periods in which additional inflorescences appeared on one or more plants already in flower (broken bars). The graphs are best interpreted in conjunc-

tion with table 3, which shows the percentage of plants in each population which had flowered by the end of the season. Thus one can properly weigh the significance in figure 7 of the flowering season of strain 11 in the 9-hour series in 1942 against the 2% of the population represented (one individual), as compared with the flowering of the same strain on 16-, 20-hour, and N photoperiods represented by 16, 12, and 40% of the respective populations.

Table 3 indicates the great variation among strains as to percentages of the populations which flowered and also shows that some of these percentages were very low. Examination of the table as to trends in the percentages, both vertically and horizontally, and attention to those strains and treatments which failed to flower, suggest that a distinction can probably be drawn between percentages which were low because of the crowded condition of the plants and those which were low chiefly because of the inhibiting effect of a certain photoperiod on the flowering of the different strains. In this connection, those populations which began to flower earliest (table 2), when the plants were small, such as strain 1 in the 9- and 13-hour series and strain 12 on N, 16-, and 20-hour photoperiods, had the highest final percentages of plants in flower as compared with many of the other strains which were delayed in first flowering until the plants were much larger.

STRAIN 1.—Ten plants of strain 1 exerted inflorescences in three of the four series on natural photoperiod in the two summers (tables 3, 4; figs. 7, 8). These appeared between September 28 and October 12, when the effective light periods at Chicago (allowing for dawn and twilight) are between 12 and 13 hours, and were probably initiated not

more than a month or 6 weeks earlier under effective photoperiods not exceeding $13\frac{1}{2}$ -14 hours. The ripe seed from which this strain was grown was harvested on September 15, 1941, at San Antonio, Texas, and the corresponding inflorescences may have been initiated on photoperiods of the same effective length in late July or early August as those at Chicago in late Au-

upper critical photoperiod for flowering between 14 and 16 hours. This value may lie close to the maximum natural photoperiod to which they are subjected in their native environment in southern Texas. They are thus short-day plants, as are so many species of tropical and subtropical range (22). They flowered more abundantly and vigorously on a 13-hour than on a 9-hour photoperiod,

TABLE 4

SEASON OF FLOWERING* ON CHICAGO NATURAL PHOTOPERIOD, AS SHOWN
BY COMBINED DATA FROM FOUR SERIES IN 1942-43

Strain no.	Season in which inflorescences first appeared on individual plants	Last date on which inflorescences appeared on plants in flower if different from column 2	No. of flowering individuals represented
12.....	May 15†-August 14	September 11	49
11.....	June 23-August 18	25
10.....	May 15†-August 18	10
9.....	July 1-August 18	August 28	20
8.....	July 5-September 23	11
7.....	June 24-September 1	September 11	17
6.....	July 22-October 9	4
5.....	July 5-September 23	September 25	18
4.....	July 5-October 9	18
3.....	August 21‡-October 15	50
2.....	July 5§-August 14	4
1.....	September 28-October 12	10

* Exsertion of inflorescence from surrounding leaf sheath.

† Flowered before clipping on May 21, 1943.

‡ Dates based on experiments in addition to series of 1942-43; inflorescences appearing in August were weak.

§ Inflorescences weak and relatively sterile.

gust or early September. That the delay in flowering until the short days of autumn at Chicago is actually the result of photoperiodic conditions is more conclusively indicated by the failure of all but three plants out of 140 to flower in the 16- and 20-hour series in the two seasons. These three plants were much delayed in flowering in comparison with those on 9- and 13-hour photoperiods (table 2), and their inflorescences were few in number and relatively sterile. The data thus show rather positively that many individuals of strain 1 have an

however, although flowering first in the latter series in both years. Approximately two-thirds of the individuals in both 9-hour series (table 3) produced inflorescences during a period of 1 month (fig. 7). About half the flowering plants exerted more than one inflorescence each, the average number per flowering individual each year being 1.6. In both years flowering in the 9-hour series ceased around the end of July, although the plants remained green and were so small (fig. 2) that crowding was not a factor. This was in contrast to the re-

sponses on the 13-hour photoperiod, where nearly all plants eventually flowered (table 3) and additional inflorescences were formed more or less continuously throughout both seasons (fig. 7) by relatively large plants (fig. 3). The average numbers of inflorescences per flowering plant in strain 1 in the 13-hour series were 3.3 and 4.4, respectively, in 1942 and 1943.

STRAIN 2.—Although originating within a few miles of strain 1, strain 2 did not respond in an identical manner. It resembled strain 3 more closely in many aspects of vegetative habit on all photoperiods (figs. 9, 10). It failed to flower abundantly on any treatment, possibly because of crowding. The nature of its photoperiodic responses as to flowering are probably best shown by the data for 1942 (tables 2, 3), seven out of twenty individuals flowering in the 13-hour series between 81 and 102 days after germination, while there was no flowering on other photoperiods during the season. In 1943, however, flowering occurred in only four individuals, all on natural photoperiod. These four plants, which had not flowered in 1942, exerted eleven weak and relatively sterile inflorescences between July 5 and August 14. Seven of them appeared in the last 2 weeks of July. If these inflorescences were initiated during the long days of June and early July, these individuals are not short-day plants so far as the production of visible inflorescences is concerned. The sterile condition, however, suggests the possibility that they may have been initiated during the shorter days of the spring months and developed only slowly to the stage of exertion in the longer days of midsummer. These and other clones of this strain are now being investigated under other conditions. On the basis of the

1942 data, and from the nature of their vegetative responses, it is the writer's opinion that probably most of the individuals in this strain are intermediate or short-day plants.

STRAIN 3.—Except for less adverse effects of a 9-hour photoperiod on its growth, the responses of strain 3 from southern Arizona were somewhat similar to those of strain 1. Because of its vigorous vegetative growth on all photoperiods, the flowering of this strain was more adversely affected by crowding than were the other strains. While it did not flower in the 9-hour series in 1942, it did so in 1943. In other experiments in 1941 (23) sixty of a population of 100 plants of strain 3 flowered on an 8-hour photoperiod, although this percentage was smaller and the inflorescences less numerous and vigorous than for plants on a 12-hour photoperiod in 1941. The abundant data for the 3 years show that a photoperiod of 12-14 hours is more favorable for flowering of this strain than one of 8-9 hours, and that the upper critical photoperiod for flowering of most individuals lies between 14 and 14½ hours (23), nearly the maximum to which it is naturally subjected. A very few plants flowered on a 16-hour photoperiod in 1941.

STRAINS 4-12.—Among the other nine strains, strain 12 from North Dakota flowered most abundantly, both as to individuals and in numbers of inflorescences. It produced inflorescences rapidly on 16- and 20-hour photoperiods in 1942 and 1943 (table 2), high percentages of the populations exerting them over a 2-month period in 1942 (table 3) and a lower percentage over a 1-month period in 1943. The average number of inflorescences per flowering plant ranged from 1.2 to 2.4 in the three series. In the four series on natural photoperiod in the

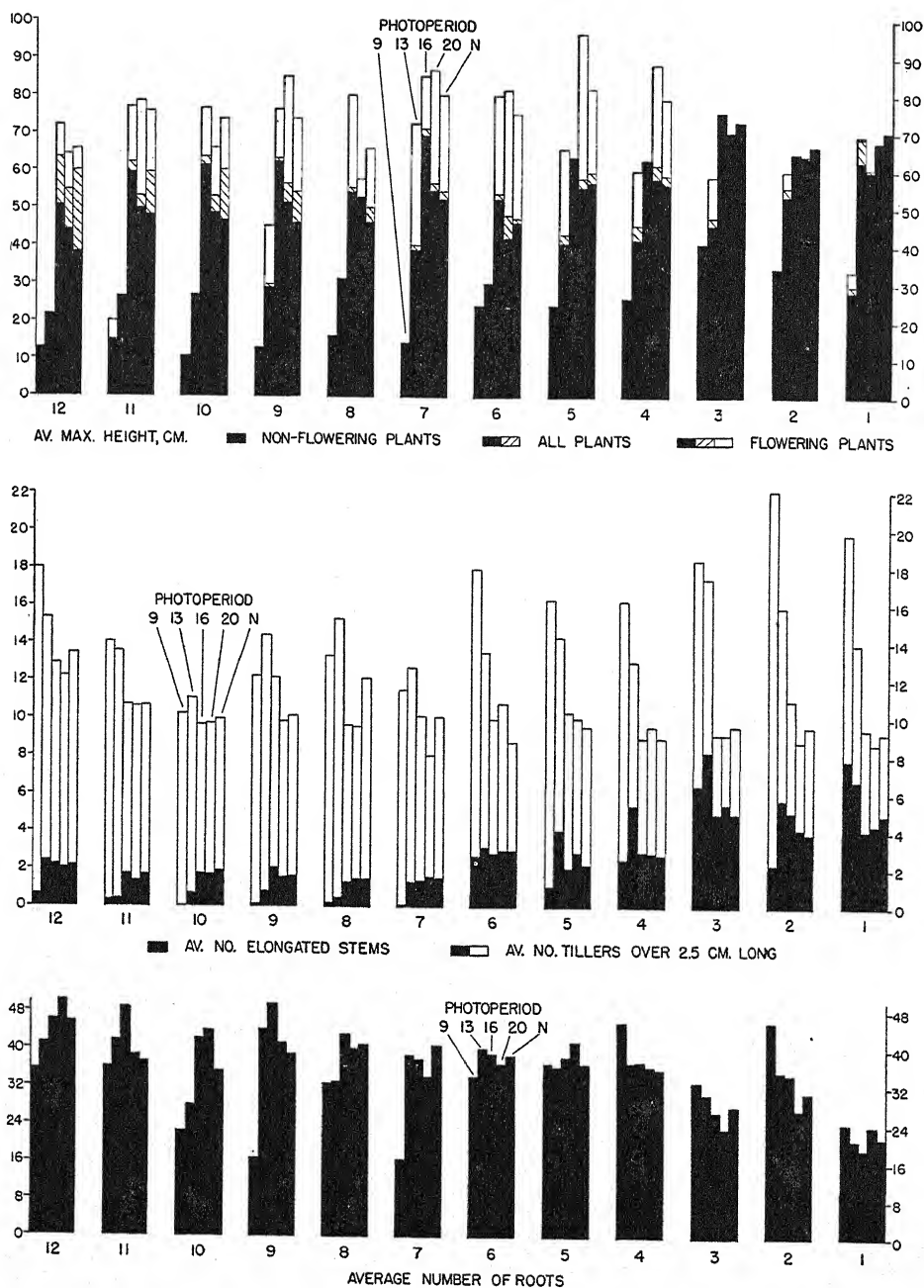


FIG. 9.—Average growth per plant of twelve strains of side-oats grama harvested in late September, 1942, after germination on April 11, 1942; grown on five photoperiods (hours) as indicated. Left to right (strains 12-1) the latitudinal sequence is north to south.

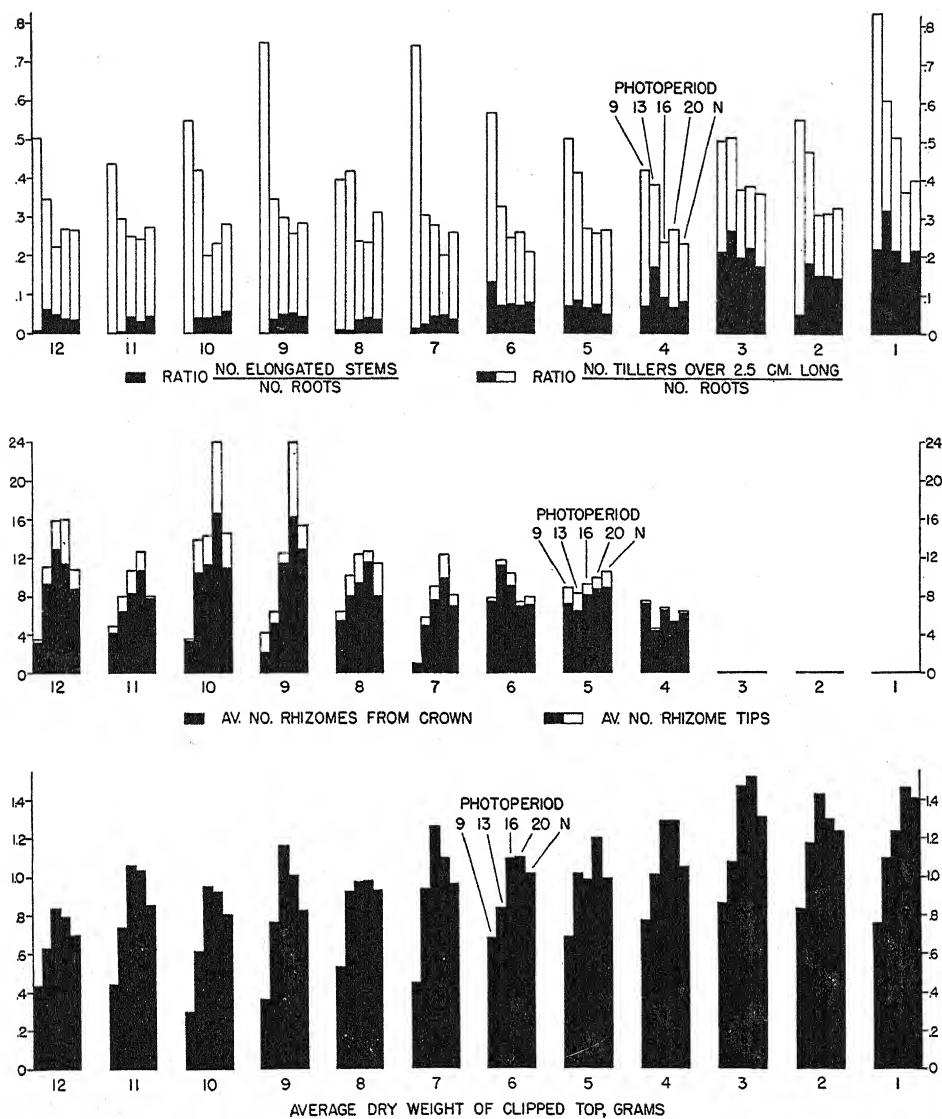


FIG. 10.—Average growth per plant of twelve strains of side-oats grama harvested in late September, 1942, after germination on April 11, 1942; grown on five photoperiods (hours) as indicated. Left to right (strains 12-1) the latitudinal sequence is north to south.

2 years, inflorescences became visible between May 15 and September 11 (table 4), although the last plant to come into flower showed its first inflorescence on August 14, and it was the only plant exerting additional inflorescences after that date (fig. 8). Practically all these inflorescences must have been initiated between late April and the end of July, during which the effective natural photoperiods at Chicago exceed 14 hours. No flowering occurred in the 9- and 13-hour series in 1942, and only three plants flowered on these photoperiods in 1943. As mentioned earlier, the latter exerted only one sterile inflorescence each, which may well have been initiated in May on natural photoperiod and escaped clipping on May 21. These plants had exerted inflorescences in May before they were clipped. The flowering data thus indicate that strain 12 consists largely of long-day plants with a lower critical photoperiod of about 14 hours. This is well substantiated by the nature of the vegetative responses on shorter photoperiods.

The flowering seasons of the other eight strains (4-11) indicate that many of them may also consist largely of long-day plants, as shown by their failure to flower in the 9-hour series, while numerous individuals within these strains flowered on 16- and 20-hour photoperiods. Flowering in the 13-hour series, especially of the northern strains, was much more limited than on the longer light periods. The data for plants on natural photoperiod (see especially table 4 and the N-N series in fig. 8) suggest that the length of the lower critical photoperiod for flowering of the "late" plants of each strain decreases with decrease in latitude of origin in the nine northern strains. While apparently some plants in all these strains can initiate and

mature inflorescences on the longest days of midsummer at Chicago (and on 16- and 20-hour photoperiods), the flowering season was progressively extended into late summer and autumn with decrease in latitude of origin of the various strains. Thus in strains 4, 5, and 6 some plants exerted their first inflorescences as late as the last week of September or early October. Some plants in these three strains also formed inflorescences on a 13-hour photoperiod. Thus, among the nine northern strains, the northern ones each seem to show more uniformity in photoperiodic response than the southern ones. It is even possible that strains 4, 5, and 6, for example, include both intermediate and long-day plants, although probably no short-day or day-neutral ones, since no plants of these strains flowered in the 9-hour series. Clonal divisions of "early" and "late" individuals of some strains are now being studied to determine more accurately their critical photoperiods and the exact status of the strains in photoperiodic classification. In general, it seems that many individuals in all twelve strains are probably able to flower most vigorously on photoperiods similar to those of the native environments in which they normally flower. The chief exception to this statement arises from the rather similar responses of some plants of the nine northern strains in the 16- and 20-hour series. Nowhere within its area of distribution is the species subjected to a natural photoperiod of 20 hours.

HOPKINS (19) grew nine strains at Hays, Kansas (approximately latitude 39°), under nursery conditions, all originating between the 33d and 47th parallels, and thus directly comparable only with strains 4-12 of the writer. In their second year, all of HOPKINS' strains be-

gan to flower in May and June, but apparently two of the southern strains showed most variation. His "Texas and Oklahoma plants (latitudes 35° and $36\frac{1}{2}^{\circ}$) started to flower on May 19th, but were not fully headed out until June 20th while the northern plants started on May 25th and were well in head by June 4th." He did not state how long flowering continued but reported that all plants were in full head on July 22. All his strains thus included individuals capable of maturing and probably of initiating inflorescences on photoperiods in excess of 14 hours, the effective photoperiod at Hays exceeding this value when the plants resumed growth in the spring. In their first year of growth, after planting on June 4, eight of his strains showed a more or less progressive lag in onset of first flowering (from July 17 to August 20) with decrease in latitude of origin (47° to 35°), comparable with the similar lag among the nine northern strains of the writer (fig. 7). His results thus confirm some of the conclusions of this study but shed no light on the most puzzling point—the differences between strains 1-3 and strains 4-12, since he had no material from the low latitudes of the first group.

ROGLER (26) reported that strains from Oklahoma, Texas, New Mexico, and Arizona (exact localities unspecified), grown in the nursery at Mandan, North Dakota (approximately latitude 47°), were so late in maturity that they were still vigorously growing at the time of the first autumn frosts, while northern strains had become dormant several weeks earlier. He gave no data on flowering seasons. Similar responses were reported by HOPKINS (19) at Hays, Kansas. The differing behavior of northern and southern strains may undoubtedly be attributed in part to the differing

effects of the decreasing photoperiod upon internodal elongation, flowering, and other growth responses. In their native environments, the northern strains have become adjusted to a short growing season of long days, frost occurring while days are still fairly long, while the southern ones occur in areas with a long frost-free season, with no likelihood of frost until the short days of late October or November, or even later.

GROWTH IN HEIGHT

Maximum height per plant was measured periodically during the summer of 1942, and on all plants harvested in September, 1942, from the crown or cut stem ends to the tip of the longest outstretched leaf or inflorescence. The same trends in height growth soon became obvious in 1943, and no measurements were made. Final heights of all strains on all treatments when harvested in September, 1942, are shown by bar graphs of the averages for flowering, nonflowering, and all plants (fig. 9). The values for nonflowering plants were naturally considerably lower than for flowering plants in any one strain and treatment.

Height growth in all strains was least on the 9-hour photoperiod. In September, 1942, the six northern strains ranged from 10.5 to 16.2 cm. tall in this series, strains 4-6 from 24.0 to 26.1 cm., while strains 1-3 were much less adversely affected by the short photoperiod, strain 3 being the tallest (40.7 cm.). In the 13-hour series, except for strain 6, height was inversely correlated with latitude of origin, whether the average of all or only of nonflowering plants is considered, ranging from 21.8 cm. for strain 12 to 68.7 cm. for strain 1 (all plants). On 16-hour photoperiod, maximum heights of the different strains were more nearly

equal. The all-plant averages of eight strains (1, 2, 4, 5, 9, 10, 11, and 12) were between 60 and 65 cm. Strain 3 was tallest (75.7 cm.) and strain 6 shortest (53.7 cm.). In only strain 12 was the average based on nonflowering plants more than 2 or 3 cm. below that based on all plants. Height values on 20-hour photoperiod tended to be slightly lower than in the 16-hour series. Northern strains were relatively much taller in both 16- and 20-hour series compared with their heights on 9- and 13-hour photoperiods than were southern strains. In general, the range of other vegetative differences among these four treatments of one strain also increased with increase in latitude of origin of the strain (figs. 2-5). Height growth in the N series resembled that of the 16- and 20-hour series, but the final values were somewhat less in the six northern strains in the N series than on 16- and 20-hour light periods, probably because of the effect of the decreasing length of natural photoperiod in late summer on the activity of the apical meristem and on internodal elongation.

Periodic measurements through the summer showed that the nine northern strains reached their maximum heights in the 16-, 20-hour, and N series soon after August 1, or increased only slightly thereafter, whether flowering or nonflowering. Many vegetative culms on strains 1-3 in the same series, however, continued to elongate and form additional large leaves until they were harvested in late September. Strains 1-3 on the 9- and 13-hour series attained their maximum heights in the first 2 weeks of July, while in the more northern strains on these photoperiods height growth continued much later, although the latter plants were still very short when harvested. This contrasting be-

havior probably resulted in part from the short-day and long-day characteristics of the two groups of strains. In the long-day northern plants the longer photoperiods, initially most favorable for culm elongation and rapid growth in height, soon result in reproductive activity and a cessation of further height growth. In nonflowering plants or culms of these strains on long photoperiods there also seems to be a cessation of activity by the apical meristems when the culms reach a certain length, although the meristems apparently remain alive. In contrast, on the short photoperiods unfavorable for flowering in the northern strains many apical meristems remain active through the season, laying down new leaves rapidly and continuously, even though practically no internodal elongation occurs and the leaves are small when mature (figs. 2, 3). Rosettes are thus formed, but there is also a continuous though limited growth in height. More than twenty-five leaves were formed on many of the primary axes in strain 12 in the 9- and 13-hour series, although the average length of these shoots (to tip of longest leaf) was only 5.2 and 7.1 cm., respectively. This was in contrast to an average length of 63.1 cm. in the 16-hour series, the latter plants bearing approximately ten leaves per primary axis. In the short-day southern strains the short photoperiods favor early—although limited—internodal elongation, but the vegetative activity of the apical meristems is soon restricted. Some initiate inflorescences, and height growth ceases, while the final activity of others results in small leaves and short internodes. The long photoperiods allow maximum internodal elongation and the continued although not especially rapid formation of large leaves in strains 1, 2, and 3.

BEHAVIOR OF PRIMARY AXIS

Tillers attained greater heights than the primary axis on nearly all plants of strains 4-12 in the 9- and 13-hour series and in strain 2 on 9-hour photoperiod. In strains 4-12, lengths of the primary axis were inversely correlated with latitude of origin in these series and were about one-half to one-third as long as the average tallest tiller per plant. In the 16-, 20-hour, and N series, the primary axes were equal to or taller than the tillers in most plants of all strains, as also in strains 1 and 3 in the 9- and 13-hour series and strain 2 in the 13-hour series. Nonflowering primary axes were alive throughout on nearly all plants in the 16-, 20-hour, and N series when harvested, but the upper portions of some had died on the shorter photoperiods, especially in the 13-hour series in all strains and in strains 1, 2, and 3 in the 9-hour series.

The effect of different photoperiods on the behavior of the primary axis is very striking, both among the strains and among the individuals of one strain. It is possible that correlations might be worked out which would make it possible to use the early behavior of the primary axis in the early selection of seedlings for "earliness" or "lateness," although no such attempt has been made in this study. Table 5 gives the percentages of plants in each strain and treatment which had produced one or more elongated internodes on the primary axis at the end of the first season. While internodal elongation is not necessarily correlated with reproductive activity in grasses, as is sometimes assumed, in some it is the first obvious sign of such initiation and may indicate conditions favorable for it. Nearly all plants of all twelve strains showed elongation in the

N, 16-, and 20-hour series, as did strains 1-3 on the shorter light periods. In the six northern strains these final values on the 16-, 20-hour, and N photoperiods were attained at successively later dates in the summer with decrease in latitude of origin, even though some plants in all strains showed elongation by June 26. Final percentage values in these series were obtained for strains 1, 2, 3, and 12 by July 15, and for strains 4, 10, and 11 by July 29. The period of time over which individual plants began elongation was thus longer for the central than for the extreme northern or southern strains, suggesting the greater variability within the former, also indicated by the longer duration of their flowering seasons on natural photoperiods. The final percentages for the 13-hour series in table 5 also indicate such variability and suggest that the percentage of plants in each strain which have a critical photoperiod of less than 13 hours for elongation of the primary axis increases with decrease in latitude of origin.

The number of elongated internodes per primary axis, based only on plants possessing them, was inversely correlated with latitude of origin in the 13-hour series in strains 4-9 (5.02-1.33, respectively). The same measurement was positively correlated with length of photoperiod within all strains in the 9-, 13-, and 16-hour series. It is probable that the failure of internodal elongation in many plants of strains 4-12 on the short photoperiods was due to unfavorable photoperiod and not to other factors, such as crowding, which was of little significance in these series. The variability in internodal elongation thus shown within strains on the 13-hour series (table 5) is probably due mostly to genetic rather than to environmental causes. Strains 1, 2, and 3 were distinct in their

behavior, practically all plants showing internodal elongation on all treatments, although the final 100% values were attained earlier in the 9- and 13-hour series than in the others. It is obvious that internodal elongation of the primary axis does not indicate reproductive activity in these three strains.

TABLE 5
PERCENTAGES OF PLANTS WITH ELONGATED
INTERNODES ON PRIMARY AXIS
SEPTEMBER 23, 1942

STRAIN NO.	PHOTOPERIODS (HOURS)				
	9	13	16	20	N
12.....	0	0	100	100	98
11.....	0	0	94	96	98
10.....	0	0	100	90	100
9.....	0	4	100	86	94
8.....	0	10	90	100	90
7.....	0	16	88	86	88
6.....	35	80	100	95	100
5.....	14	86	94	96	100
4.....	16	86	96	94	100
3.....	100	100	100	100	100
2.....	95	100	100	100	100
1.....	100	100	100	100	100

TILLER DEVELOPMENT AND CULM ELONGATION

Total numbers of tillers and tiller buds over 2.5 cm. long were counted on plants harvested in late September, 1942 (fig. 9). In strains 7-10 the greatest numbers were formed on the 13-hour series, followed by the 9-hour series, while in strains 1-6 and 11 and 12 the values were inversely correlated with length of photoperiod in the 9-, 13-, and 16-hour series. In most strains, values for 20-hour and N series were near those of the 16-hour series. In all strains, basal branching was thus favored by the shorter photoperiods, most strikingly in strains 1, 2, and 3, somewhat less in 4, 5, and 6, and moderately in 7-12.

Averages based on either flowering or nonflowering plants were very near those based on all plants, although in most strains and treatments the average for flowering plants was greatest.

On the shorter photoperiods, especially in the 9-hour series, numbers of tillers tended to be inversely correlated with latitude of origin, except in strains 11 and 12, which had higher values than some of the strains originating south of them. Differences in tiller number among strains on longer photoperiods were not statistically significant, except for the somewhat higher values in strains 11 and 12 in the 16- and 20-hour series. On natural photoperiod, tiller numbers in strains 8 and 12 were significantly greater than in the other strains, which showed little difference. With respect to different strains, these experiments thus show that only strain 12 may be considered to tiller relatively more profusely than any other strain on all treatments, in spite of the inverse correlation between latitude of origin and tiller number in the 9-hour series. In nursery-grown plants at Hays, Kansas, HOPKINS (19) reported greater numbers of tillers on southern than on northern strains, but he did not define a "tiller" as counted.

Tillers with one or more elongated internodes were counted in the plants harvested September, 1942. The surrounding leaf sheaths were stripped away to verify the elongation. All plants of strains 1, 2, and 3 in all series and nearly all plants of strains 4-12 on 16-, 20-hour, and N photoperiods had one or more tillers showing such elongation. In strains 4-12 in the 13-hour series, the percentages of plants showing elongation were 98, 92, 80, 52, 25, 36, 40, 22, and 66, respectively, while in the 9-hour series the values were 60, 22, 75, 8, 15, 10, 0, 12, and 26. Thus in the six northern

strains (except for strain 12, 13-hour series) less than half the plants on the two shorter photoperiods showed internodal elongation, and this was often found only in a limited number of tillers per plant showing slight elongation (a few millimeters) of one or more internodes. The inhibiting effect (or lack of stimulation) of the short photoperiods on internodal elongation and correlated limitations on foliage growth in the northern strains probably account in part for their growth being less vigorous than that of southern strains in nurseries in southern latitudes in the United States (27). They are there subjected to maximum photoperiods shorter than those optimum for their most vigorous growth.

The average numbers of elongated stems per plant, based on all plants, were not significantly different within strains 7-12 among the 16-, 20-hour, and N series, nor among these strains within any one of these series, except for the slightly higher values in strain 12 (fig. 9). The numbers of elongated stems were significantly higher in strains 4-6, and even more so in strains 1-3, on the long (16- and 20-hour) and N photoperiods than in more northern strains. Except in strains 1-3, the values for plants on the short photoperiods shown in figure 9 reveal no consistent trends, as they are based on all plants and are thus influenced by the different percentages of plants which showed elongation. Flowering plants consistently had greater numbers of elongated stems than had nonflowering plants in all strains and series.

The percentage of tillers showing elongation is a more useful comparative index among strains and treatments than the total numbers of elongated stems. In strains 1-3 on 16-, 20-hour,

and N photoperiods, the former value was between 41 and 59%, in strains 4-6 between 20 and 35%, and in strains 7-12 between 12 and 20%. In the 13-hour series the percentages were 49, 36, 50, 41, 29, 24, 11, 3, 5, 6, 3, and 16 for strains 1-12, respectively; in the 9-hour series the sequence from south to north was 39, 11, 35, 15, 7, 15, 1, 2, 2, 0, 2, and 4%. Thus there is some tendency toward an inverse correlation between latitude of origin and percentage of tillers elongating in all the series, especially when the strains are grouped as 1-3, 4-6, and 7-12. This suggests that the northern strains are probably actually less vigorous in growth than the southern ones under many types of environment. Northern strains have been reported to be less vigorous than southern ones in several nurseries in different latitudes (13, 19, 26, 27).

NUMBERS OF ROOTS AND RHIZOMES

One pot of each strain from each series was washed out in September, 1942, and the numbers of crown roots and rhizomes counted (figs. 9, 10). While the sample was probably inadequate, certain tendencies are suggested. Strains 1-4 had their highest numbers of roots in the 9-hour series, while among strains 6-12 this series had the lowest numbers. In strain 5 differences among series were slight. Numbers of roots in the four southern strains tended to be inversely correlated with length of photoperiod, while in the seven northern strains the correlation was direct. In all except the 9-hour series, the three southern strains (especially strain 1) had smaller numbers of roots than most of the other strains on comparable treatment.

The ratio of number of tillers over 2.5 cm. long/number of roots was highest

on the two short photoperiods in all strains and tended to be inversely correlated with length of photoperiod in the 9-hour, 13-hour, and 16-hour series. The ratio of number of elongated stems/number of roots was greatest in strains 1-3 because of the greater number of elongated stems and the smaller number of roots in those strains.

Rhizomes were developed on the nine northern strains only (fig. 10). Their absence on strains 1-3 again suggests that these three strains form a group apart from the others. In strains 4-12 rhizomes developed on practically all plants in the first summer, and when harvested in September some of them, in all except the 9-hour series, had sent up green shoots with leaves up to 12 inches long. The horizontal rhizomes tended to branch at varying distances from the crown, most markedly in strains 7-12 (fig. 10). In these strains the numbers of rhizomes from the crown tended to be directly correlated with length of photoperiod, while relationship to photoperiod was not obvious in strains 4-6. In strains 7-12 rhizome development was thus favored by the longer photoperiods, while tiller formation was favored by the shorter light periods, so that the ratio of tillers/rhizomes was inversely correlated with length of photoperiod. This latter relationship was also true in strains 5 and 6.

TOP WEIGHT

Dry weights of tops as clipped in September, 1942, were determined for all plants (fig. 10). There were positive correlations between dry weight and length of photoperiod for all strains in the 9-, 13-, and 16-hour series. Weights of plants on the 20-hour treatment were near those on 16-hour. Southern strains weighed more than did the northern ones within

all series, suggesting again that the former are inherently more vigorous. There was a tendency toward an inverse correlation between weight and latitude of origin within any one series. Similar results have been indicated for nursery-grown plants on natural photoperiods (27). HOPKINS (19), at Hays, Kansas, reported that at the end of the first year of growth southern plants had produced approximately twice as much air-dry forage as had those from central latitudes and four times as much as had those from the north. A greater proportion of the weight was contributed by stem tissue in the southern strains than in the northern. This was also true in the experiments here reported, so that differences in weight of palatable forage would have been somewhat less than differences in total weight.

Discussion

This study has confirmed the reports of others that plants of side-oats grama from different latitudes are morphologically and physiologically distinct when grown together under similar experimental conditions. This diversity must therefore have a genetic basis. Each strain in turn shows considerable genetic differentiation. The study has further shown that this diversity involves adaptation to different photoperiods by the various strains. Plants of three strains from southern Texas and southern Arizona, in photoperiodic experiments, show most nearly normal (nearest to that under field conditions) vegetative and flowering behavior on a photoperiod (13 hours) close to those of their native growing season. Most of them fail to flower, although they grow luxuriantly, on photoperiods longer than 14 hours. They are not naturally subjected to such long photoperiods. Under nursery conditions

in more northern latitudes most of the individuals in these strains are thus delayed in flowering until the shorter days of autumn, when they are likely to be injured by frost, the latter often occurring while they are still in a vigorous vegetative condition. Under the conditions of this experiment these strains were also non-rhizomatous. In North Dakota and Kansas, southern strains are also prone to winter injury (19, 26). It is obvious, therefore, that they are not genetically favorable for acclimatization in latitudes very far north of their native environments, unless their vigorous erect vegetative habit would make it desirable to attempt selection of cold-resistant individuals capable of flowering on long photoperiods. Strains 1 and 3 include a very low percentage of plants able to flower on a 16-hour photoperiod. Selections desirable for use as hay could be made from these three strains.

A North Dakota strain, in contrast, consists of individuals showing normal (nearest to that under field conditions) vegetative and flowering behavior only on photoperiods of 14 hours or longer (long-day plants). In their natural growing season they are not subjected to photoperiods of much lower value. When grown on shorter photoperiods, most of them do not flower or show much internodal elongation, although they tiller abundantly to produce rosettes. Rhizomes are formed on the shorter photoperiods, but not so abundantly as on longer ones, and dry weight yields are also lower. When grown in southern latitudes in the United States, where maximum photoperiods do not exceed 14 hours for more than a few weeks, such a strain could not compare favorably with those from more southerly origins, irrespective of the suitability of other environmental conditions.

Strains from central latitudes in the United States, especially those from Oklahoma, show considerable variation in photoperiodic response within strains, although these responses tend to exhibit a gradient among strains correlated with latitude. Strains from Nebraska and Kansas apparently consist mostly of long-day plants but probably with shorter critical photoperiods than those from North Dakota. Strains from Oklahoma and New Mexico certainly include some long-day individuals and probably some which are intermediate in their photoperiodic requirements; that is, which flower only on an intermediate range of photoperiods, probably of lengths corresponding with the range to which they are naturally subjected in their growing seasons. Vegetative growth of these strains of central latitudinal origin is less adversely affected by short photoperiods with decrease in latitude of origin, while all exhibit luxuriant vegetative growth on long photoperiods. The strains from Oklahoma and New Mexico, because of their apparently greater genetic diversity, and behavior resembling that in the field over a wide range of photoperiodic conditions, would probably be best fitted—from the standpoint of photoperiodic requirements—for acclimatization to latitudes north or south of their own if they showed other characteristics making such introduction desirable. Within their own latitudes they show most promise for the selection of "early" and "late" individuals, so far as this behavior is correlated with photoperiod. The use of artificial photoperiodic control in such selection would be of value in breeding programs.

The different strains of side-oats grama in these experiments have exhibited practically the entire range of reported photoperiodic responses, and

as a species it cannot be classified photoperiodically except with reference to strains. This is striking evidence that it is unwise to attempt such classification when only a few plants have been observed, although many species have been listed as long-day, short-day, or day-neutral plants on the basis of the responses of one or a few clones. Such generalizations at the species level can be safely made only when strains from a wide latitudinal range have been investigated.

These experiments also show that the habit of a strain in one environment is not predictable from its mode of growth in another, as has been conclusively demonstrated by TURESSON (32, 33, 34) and CLAUSEN, KECK, and HIESEY (7). These investigators showed that genotypic differences not apparent in one environment were often revealed in another. The range of vegetative expression induced in one strain by the different photoperiods was greater in the northern than in the southern strains, and strain differences of a vegetative nature were accentuated by the shorter photoperiods. This is in contrast with the results of WERNER (36), who found that a simulated "northern" environment (longer photoperiods as one factor) gave opportunity for manifestation of more varietal differences among several varieties of potatoes than did a "southern" one. It also contrasts with the observations of CLAUSEN *et al.* (7), who found in their reciprocal transplant experiments that "ecotypical differences may be accentuated at one station over another, and the most vivid impressions of these differences are obtained at that garden where the species [*Potentilla glandulosa*] as a whole does its best." The first but not the latter part of their statement

applies to the growth of side-oats grama in these experiments.

The wide range of morphological expression of the different strains induced by the various treatments casts some doubt on the general validity of the five biotypes of side-oats grama described by FULTS (13), based on plants from different latitudinal sources grown in one nursery of central latitude. It is problematical whether his descriptions would apply accurately to the same plants grown in nurseries in the northern or southern United States, where natural photoperiods of different length might accentuate or diminish the differences between individuals assigned to one or another of his biotypes.

There is little doubt that side-oats grama originated in low latitudes, possibly in southwestern United States or Mexico, and has spread north and south to higher latitudes in Argentina and southern Canada. The center of distribution of the genus is in Mexico and southwestern United States (16). Other species in the same subgenus (*Atheropogon*) are confined to low latitudes (under 35°), and plants in the genus with low chromosome numbers have been reported chiefly from this region (13). Most of the investigated individuals of side-oats grama are hexaploids ($2n = 42$), but FULTS (13) also reported chromosome numbers of 28, 35, 40, 45, 56, 70, and 98 in the species. The individual with $2n = 28$ was from Kansas, while the few plants with numbers higher than $2n = 42$ were from Oklahoma, Texas, Arizona, and Colorado. FULTS stated that "the production of new types through various chromosomal aberrations is probably still a dynamic process in this region," and that "it is likely that the diploid type of $2n = 14$ will be found by further

search, especially in the southwestern biotypes." FULTS' first statement may help explain in part the genetic variability of the writer's material from Oklahoma and New Mexico, although no chromosome counts have been made upon it. His data thus suggest, although they do not confirm, an origin and continuing evolution of side-oats grama in low latitudes, but apparently the hexaploid complex has been most involved in the spread to higher latitudes in the United States.

The present photoperiodic differentiation of the species is thus of interest in connection with its probable origin and evolution. The typical short-day response of the three southern strains could represent the original adaptation to the short photoperiods of low latitudes in which the species originated and in which these strains persist. Within these strains, however, a few individuals (possibly day-neutral, although clonal studies on different photoperiods are not completed) are able to flower on longer photoperiods. While of little importance in adjustment of these strains to their present environments, they suggest that races with similar ability, however it originated, would have been perpetuated through natural selection in a migration to higher latitudes in which flowering must necessarily occur in the long days of the frost-free season. Even with the development of the rhizome habit, it is probable that plants capable of flowering only on short photoperiods would have been eliminated or very restricted in their rate of migration if they persisted through vegetative propagation. The populations spreading north thus would have come to consist largely of day-neutral plants, or of intermediate or short-day plants with a very long criti-

cal photoperiod. Some of the "late" plants from Oklahoma and New Mexico may be of the latter type, but it is doubtful whether many individuals in these strains are day-neutral. The mode of evolution of the typical long-day response of the more northern strains is obscure. The ability to flower on a 20-hour photoperiod is not of survival value at present, and it is doubtful whether the species has ever ranged to latitudes where such photoperiods are encountered. Any complete evolutionary history of the species must also account for a loss of ability to flower on short photoperiods by the northern strains and their tendency to limited growth and dormancy as photoperiods decrease below a certain level. But whatever the mechanism of the evolutionary process back of such diversity, it has certainly resulted in a high degree of adjustment by the respective strains to the range of photoperiods encountered in their native environments during the season of the year otherwise favorable for growth. The ability of some individuals in most of the strains to grow and flower well over a wider range of photoperiods, while of no importance in their native environments, has undoubtedly been of evolutionary significance and of survival value in the migration of the species to different latitudes. Such variants hold most promise for acclimatization to areas north or south of their native ones and possibly for the ultimate segregation of desirable day-neutral types.

Summary

1. Twelve strains of side-oats grama (*Bouteloua curtipendula*), originating over a latitudinal range of approximately 17° from San Antonio, Texas, to Cannonball, North Dakota, have been grown

for a 2-year period in the greenhouses at the University of Chicago. Their responses on Chicago natural daylength and on 9-, 13-, 16-, and 20-hour photoperiods have been analyzed.

2. Vegetative and flowering responses on the different photoperiods showed that three strains from southern Texas and southern Arizona consist almost entirely of intermediate or short-day plants, with an upper critical photoperiod for flowering between 14 and 16 hours. These strains flowered more vigorously on a 13-hour than on a 9-hour photoperiod. A North Dakota strain consists largely of long-day plants with a critical photoperiod of about 14 hours. The other eight strains, from Nebraska, Oklahoma, Kansas, and New Mexico, also include numerous long-day individuals, although the data suggest that the length of the critical photoperiod for flowering of the "late" plants decreases with decrease in latitude of origin of these strains. The Oklahoma and New Mexico strains accordingly show more diversity in response within each strain on each treatment and may possibly include both intermediate and long-day plants. Many individuals in all twelve strains are probably able to flower most vigorously on photoperiods similar to those of their native environments on which they normally flower. Other responses indicate a similar nice adjustment to the range of photoperiods in the growing seasons of their native environments, although a few individuals in most strains grow and flower well over a wider range.

3. Internodal elongation and height growth were increasingly suppressed on a 9-hour photoperiod—and to a lesser extent on 13-hour photoperiod—with increase in latitude of origin. In the other

series, maximum heights were more nearly equal, and other vegetative differences among strains were also less apparent. The development of the primary axis was more strongly correlated with strain origin and treatment than was that of the tillers, and its early behavior might be a useful criterion in genetic analyses. Tillers in all strains were more abundant on the shorter photoperiods than in the other series, but their numbers were not strikingly correlated with latitude of strain origin. The percentages of tillers showing internodal elongation tended to be correlated inversely with latitude of origin in all series. Numbers of crown roots tended to be correlated inversely with length of photoperiod in the four southern strains, while in seven northern strains the correlation was direct. In all except the 9-hour series, the three southern strains had smaller numbers of roots than most of the other strains on comparable treatment. Rhizomes were not produced by the three southern strains. Dry weights of clipped tops were directly correlated with length of photoperiod in any one strain and more or less inversely with latitude of origin among strains.

4. These results show definitely that a widely ranging native species may be so differentiated in its photoperiodic requirements that its assignment to a particular photoperiodic class may be generally invalid unless based on material from a wide latitudinal range. The results, and the conclusions based on them, also help to explain some of the differences which have been observed among similar latitudinal strains grown together in various nurseries. They also indicate the nature of the restriction which the adaptation of strains of this species to particular ranges of photo-

period imposes upon plant breeders, who might be aided by the use of artificial photoperiodic control in their programs. The possible origin of such photoperiodic differentiation should be of considerable interest to students of evolution and plant geography, since the species probably originated in low latitudes with short days and has therefore become

secondarily adapted to the long days of higher latitudes.

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FUNGISTATIC ACTION OF DIPHENYL ON CITRUS FRUIT PATHOGENS¹

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Introduction

TOMPKINS (4) was the first to point out the value of diphenyl in controlling green-mold rot of oranges when used as impregnating material for paper wraps. When treated wraps were used, he noted that not only was the number of rotting fruits reduced but also the spore formation accompanying rotting.

Following these studies, FARKAS (1) showed from the results of extensive shipping tests with oranges from Palestine to England that the percentage of green mold (*Penicillium digitatum*) was six or seven times higher with untreated

wraps than when diphenyl-impregnated wrappers were used. His tests on the physiological effects of diphenyl also showed that if this material is taken up at all by the fruit, it remains in the peel, and the quantities concerned are so minute that they are entirely negligible as compared with the amounts that can be tolerated by man (2).

FARKAS and AMAN (3), studying the action of diphenyl vapor on various molds, found that a concentration of 0.08 mg. per liter of air stopped development of *P. digitatum*, *P. italicum*, and *Diplodia* sp. completely. However, the spores and older hyphae continued to grow when the diphenyl was removed.

During the last 3 years there has been considerable increase in the use of diphenyl-impregnated wrappers for oranges, lemons, and grapefruit by commercial firms in the United States. Although the shipping tests abroad and those made in this country have shown diphenyl to be

¹ Investigation conducted by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, in co-operation with the Department of Botany at the University of Chicago.

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effective in the control of the green and blue molds (*P. digitatum* and *P. italicum*) of citrus fruit, the manner of action of this material and its effect on spore germination and growth of these fungi have not been investigated. This paper records the results of studies of the action of diphenyl vapor on spore germination and growth in pure culture, not only of the blue and green molds but also of other of the more important citrus-rot fungi.

From among the many fungi that affect citrus fruit during transit, storage, and marketing, the following were selected for study as being the most serious pathogens: *Alternaria citri* Ell. and Pierce, *Botrytis cinerea* Pers., *Colletotrichum gloeosporioides* Penz., *Diplodia natalensis* P. Evans, *Phomopsis citri* Fawc., *Penicillium digitatum* Sacc., *P. italicum* Wehmer, *Phytophthora citrophthora* (R. E. Sm. & E. H. Sm.) Leonian, *Sclerotinia sclerotiorum* (Lib.) Dby., and *Trichoderma viride* Fr.

Preliminary tests indicated that the vapor from 0.05 gm. of diphenyl was effective in inhibiting spore germination and mycelial growth of some of the citrus-rot fungi in plate cultures and their spores in water drops; however, 0.2 gm. diphenyl was used in each test in order to assure a strong concentration of vapor.

The first method of studying the effect of diphenyl vapor on the growth of these fungi consisted in making plantings of mycelium from pure cultures on potato dextrose agar (pH 6.8). Diphenyl crystals were placed in the center of the inoculated plates in one series; a second series similarly inoculated but to which no diphenyl was added served as controls. Two plantings of the organism to be tested were made on each plate about midway between the crystals and the

edge of the plate. Since diphenyl is only very slightly soluble in water at ordinary temperature, it was found satisfactory to place the crystals directly on the agar; but in tests of each organism at least one set of cultures was made in which the crystals were placed in glass rings in the center of the plate to prevent the possibility of any surface spread or diffusion of the chemical. In tests with fungi that would make some growth at 35° F., the diphenyl vapor was effective in controlling the growth if it had any fungistatic action on the organism at higher temperatures (60°–80°). The data presented in table 1 were selected as typical of several series of temperature tests on the growth of the rot-producing fungi of citrus fruit at 40° and 70° F. The lower temperature approximates that commonly found in refrigerator cars during transit and the higher temperature approximates that found in most retail stores.

The diameters of colonies of the fungi grown on agar plates with and without diphenyl were measured at regular intervals. The growth characteristics of each organism are shown in figure 1. The degree of inhibition is shown by comparing the diameters of the colonies (table 1).

Two methods were used in studying the effect of diphenyl vapor on spore germination. In the first, suspensions of the various fungi were made by placing spores from vigorously growing, 5–10-days old cultures into tubes containing 50 cc. of sterile water and shaking vigorously to break up spore clumps. Two cubic centimeters of each suspension was transferred to another tube containing 20 cc. of sterile water. These tubes were thoroughly shaken to insure uniformity of spore distribution, after which drops of the final suspension were pipetted to cover slips which were inverted over Van

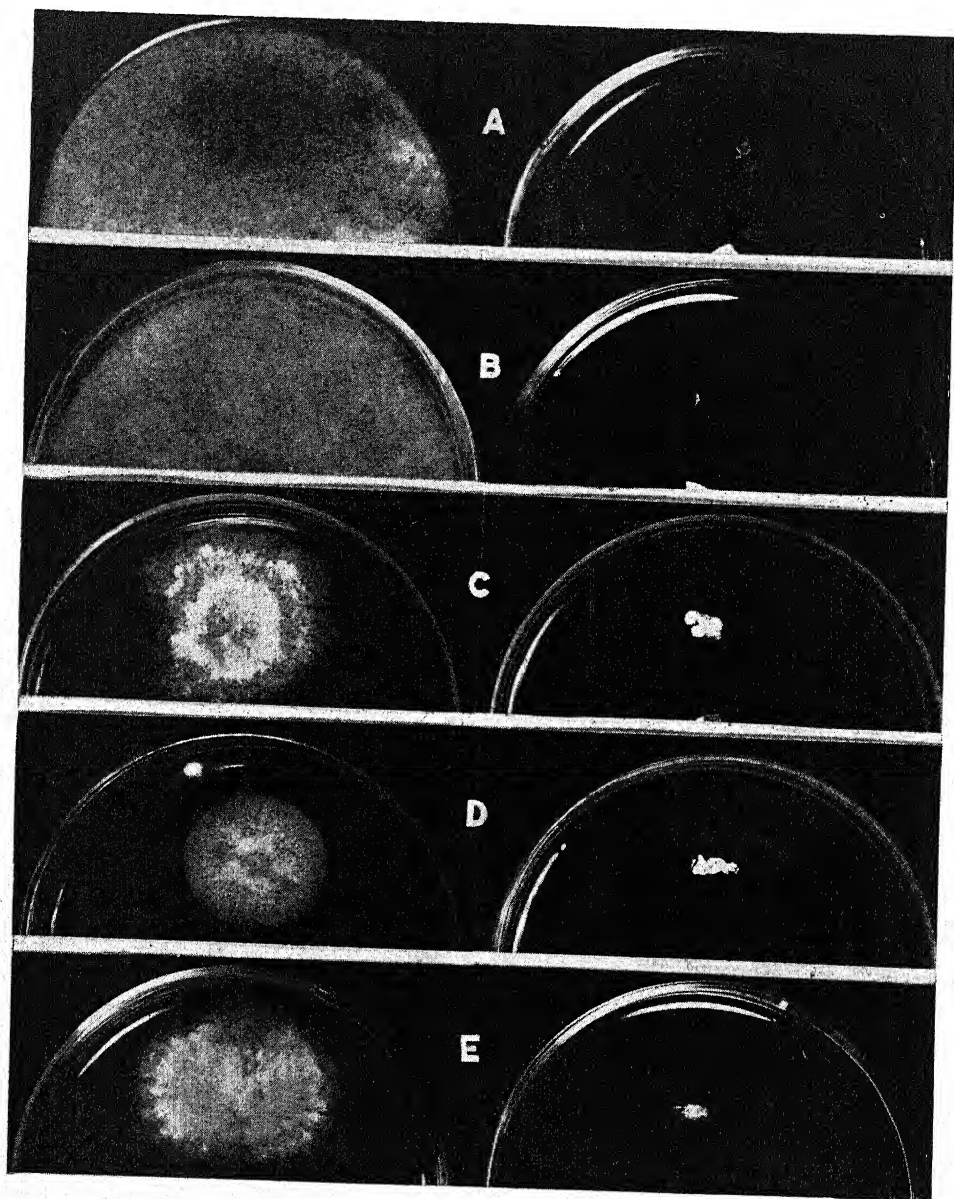


FIG. 1.—Effectiveness of diphenyl in controlling growth of citrus pathogens: left, control; right, treated. A, *Diplodia natalensis*; B, *Botrytis cinerea*; C, *Phomopsis citri*; D, *Penicillium italicum*; E, *P. digitatum*; F, *Sclerotinia sclerotiorum*; G, *Trichoderma viride*; H, *Colletotrichum gloeosporioides*; I, *Alternaria citri*; J, *Phytophthora citrophthora*. Cultures grown at 70°–75° F. and usually photographed the third day.

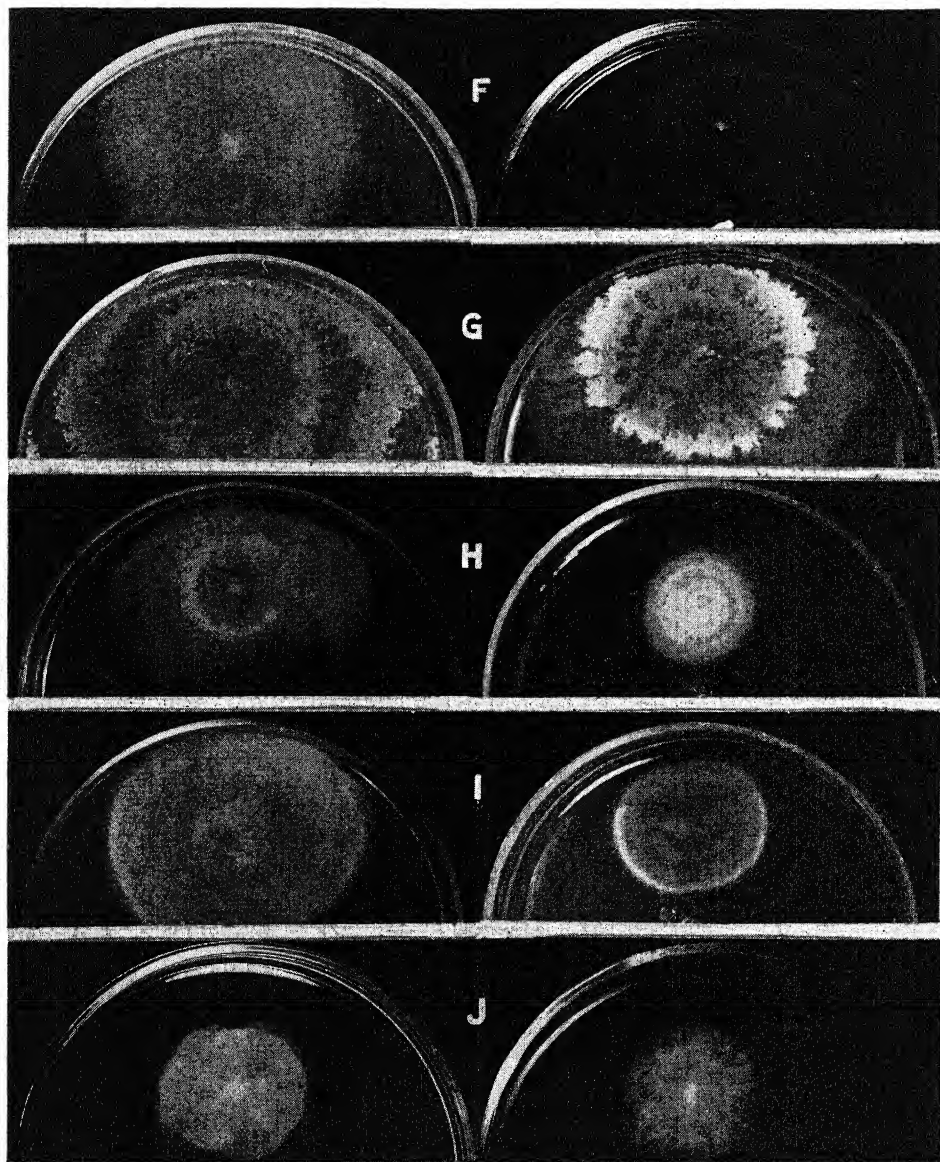


FIG. 1.—Continued

Tieghem cells affixed to glass slides. Diphenyl crystals (0.2 gm.) were placed in the bottom of these cells. Spore-containing drops inverted over Van Tieghem cells in which no diphenyl was added

spore suspension of the fungi to be studied to the surfaces of plates of potato dextrose agar by means of a sterile loop. The plates were then tilted to allow the drops to spread along the surface of the agar.

TABLE 1
GROWTH OF CITRUS FRUIT PATHOGENS ON AGAR PLATES
C, control; D, exposed to diphenyl vapor

PATHOGEN	TREATMENT	DIAMETER OF COLONIES (MM.)							
		Days at 40° F.				Days at 70° F.			
		5	7	9	21	1	3	5	7
Alternaria citri.....	C	4	8	11	25	8	35	57	76
	D	T*	2	4	18	5	20	33	46
Botrytis cinerea.....	C	5	14	31	80	7	54	80†
	D	0	0	0	0	0	0	0	0
Colletotrichum gloeosporioides.....	C	0	0	0	0	T	32	56	74
	D	0	0	0	0	T	16	27	39
Diplodia natalensis.....	C	0	0	0	0	15	80
	D	0	0	0	0	0	0	0	0
Penicillium digitatum.....	C	0	0	0	4	T	23	46	63
	D	0	0	0	0	0	0	T	3
Penicillium italicum.....	C	T	3	4	17	4	28	59	80
	D	0	0	0	0	0	T	T	T
Phomopsis citri.....	C	0	0	0	3	0	20	40	57
	D	0	0	0	0	0	T	8	12
Phytophthora citrophthora.....	C	0	0	0	0	4	23	39	57
	D	0	0	0	0	T	27	57	80
Sclerotinia sclerotiorum.....	C	0	0	0	9	T	63	80
	D	0	0	0	0	0	0	T	8
Trichoderma viride.....	C	0	0	0	T	7	68	80
	D	0	0	0	0	0	17	61	80

* Trace of growth.

† Full plate.

served as controls. Unless otherwise indicated, the cells were incubated at 75° F. All tests were made in duplicate. The percentage of germinated spores was determined by counts at the end of 24, 48, and 72 hours (table 2).

The second method consisted of the addition of two drops of the diluted

In one series a small crystal of diphenyl was added to each of the plates. A second series, similarly inoculated but receiving no diphenyl, served as controls. Observations of the progress of spore germination were readily made by placing the plate on the stage of the microscope and making direct counts.

Results

The reaction of the important citrus fruit pathogens to diphenyl vapor as shown by growth rate in plate culture and spore germination studies is shown in tables 1 and 2. It will be noted that a few fungi were totally inhibited in growth, some were checked at first and then grew moderately well later, while others were retarded only slightly. The only case of

Apparent recovery from the initial shock of the chemical was exhibited by *Sclerotinia* and *Trichoderma*. These fungi eventually grew all over the culture plates, even covering the diphenyl crystals (fig. 1G). Although the mycelium of *Trichoderma* was not quite normal, many spores were produced. The mycelium of *Sclerotinia* became cream-colored and more compact than normal. After

TABLE 2

AVERAGE PERCENTAGE GERMINATION OF SPORES OF CITRUS FRUIT PATHOGENS IN WATER DROPS AND ON AGAR PLATES, EXPOSED TO DIPHENYL VAPOR AT 75° F.

PATHOGEN	PERCENTAGE GERMINATION AFTER											
	24 hours				48 hours				72 hours			
	Control		Diphenyl		Control		Diphenyl		Control		Diphenyl	
	Water	Agar	Water	Agar	Water	Agar	Water	Agar	Water	Agar	Water	Agar
<i>Alternaria citri</i>	88	77	10	22	100	86	52	69	100	93	95	95
<i>Botrytis cinerea</i>	85	72	0	14	100	88	43	52	100	96	50	69
<i>Colletotrichum gloeosporioides</i>	81	69	30	52	83	74	85	79	83	89	85	88
<i>Diplodia natalensis</i>	85	98	3	6	100	99	7	11	100	99	12	16
<i>Penicillium digitatum</i>	10	47	6	12	50	56	13	18	62	77	17	24
<i>Penicillium italicum</i>	40	33	0	8	64	61	3	17	75	75	10	22
<i>Phomopsis citri</i>	90	79	9	20	95	84	16	39	95	88	10	46
<i>Trichoderma viride</i>	2	12	0	0	25	27	0	12	62	43	18	35

increased growth in the presence of diphenyl vapor was that found in cultures of *Phytophthora citrophthora*. In the many tests made with this fungus it always grew as fast or faster in diphenyl vapor than in the plates without this chemical. The colonies of this fungus produced in the control plates were white and appressed, with the margins sharp and regular, whereas in the diphenyl plates they were white and somewhat flocculent, with irregular margins (fig. 1J). A few apparently normal sporangia were produced within a week in the diphenyl vapor, but none were observed in the control plates under the same conditions.

several weeks' growth, a few yellowish sclerotia were observed in diphenyl-plate cultures. Apparently the ability to tolerate diphenyl vapor varies greatly with the species.

Regardless of whether growth was slightly or greatly inhibited, all the fungi showed one common characteristic reaction to the vapor. The newly developed hyphae were greater in diameter than normal and there was a great increase in the secondary branching. With the fungi that showed least growth, such as *Botrytis*, *Diplodia*, and *Penicillium* sp., there was a tendency to produce compact masses of hyphae made up of malformed or

giant cells. White masses of hyphae bearing giant cells resembling spores were particularly conspicuous in *Penicillium* (fig. 2A). No normal green or blue spores were ever produced in the presence of diphenyl. On removal of the diphenyl from such cultures, however, the mycelium began to develop normally and the

would appear that inhibition was somewhat less, in that a rather high percentage of spores germinated; but in such cases usually only short, thick, malformed germ tubes were produced and these often burst before they made much growth (fig. 2C). In the case of *Botrytis* and *Diplodia*, whose vegetative growth

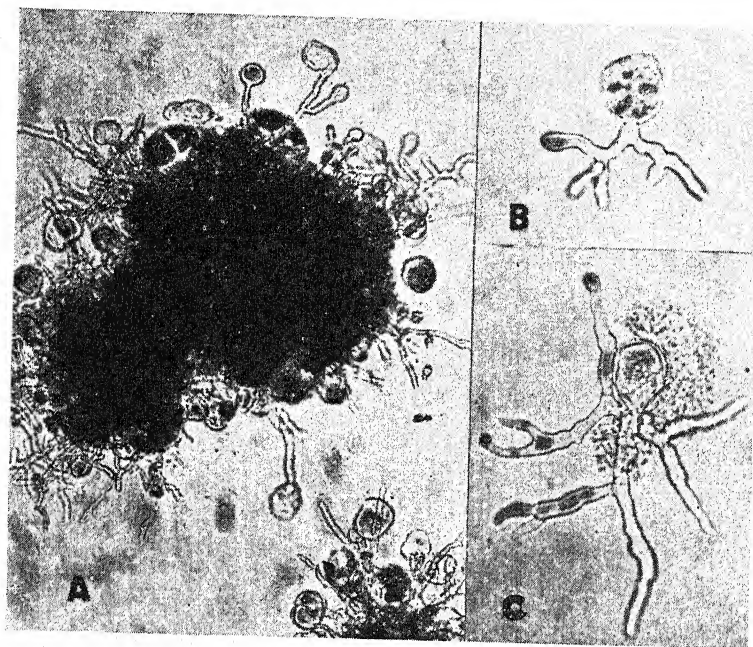


FIG. 2.—Characteristic growth of *Penicillium* and *Diplodia* in the presence of diphenyl vapor. A, giant cells and distorted hyphae of *P. digitatum*. Note lack of normal spores. Transmitted light. B, swollen spore of *D. natalensis* showing abnormal branching of germ tube. C, burst spore and contents of *D. natalensis* showing branching hyphae.

characteristic colored spores were formed within 2 or 3 days, showing that the chemical has a fungistatic rather than a fungicidal action. None of the fungi tested in pure culture have been killed by the vapors, even in concentrations many times stronger than necessary to inhibit growth.

The spore germination studies reflected about the same reactions to diphenyl vapor as were found in the agar-plate cultures. In a few instances it

on agar plates is completely inhibited in the presence of diphenyl, the spores germinated moderately well but their germ tubes stopped growth when they reached a length equivalent to about two spore diameters (fig. 2B). Spores of *Diplodia* frequently became enlarged and distorted and then burst before germ tubes were developed, but *Botrytis* spores did not burst. The bursting of spores and germ tubes and the prolific branching of hyphae suggest that the diphenyl vapor

affects the plasma membrane in such a manner as to interfere with its regulatory powers, although death of cells does not occur except when they actually burst.

The control of rot of citrus fruit during transit, storage, and marketing by the use of diphenyl-treated wraps is evidently due to the inhibition of germination of contaminating surface spores, the retardation of growth of exposed hyphae already present on the fruit, and the prevention of new spore formation that often is the source of infection during handling.

Diphenyl-treated paper wraps are now successfully used for control of decay in many commercial shipments of citrus. During the course of this investigation another method of using the diphenyl as a fungistatic agent was demonstrated. Fiberboard boxes, such as are sometimes used for shipping apples, were fitted with paper-pulp trays containing twenty cuplike depressions for holding individual fruits (the Friday pack). Five trays were packed into each box. In one experiment 100 oranges from an apparently healthy lot of fruit were placed in trays impregnated with diphenyl and 100 were placed in untreated trays. The fruit in the treated trays was packed in one box and sealed, and the control fruit in untreated trays was packed in another box and sealed. At the end of a storage period of 3 weeks at 72° F. and relative humidity of 40%, the control box of fruit had 25% decay and the fruit in the treated trays had 1% decay. Figure 3 shows green mold rot in a treated and in an untreated tray. The vapors from the treated trays showed a fungistatic action on the rot-producing organisms such as was demonstrated in the pure culture studies.

Summary

1. The effect of diphenyl vapor on the growth of ten citrus fruit pathogens was studied. Growth of a few organisms was totally inhibited, some were moderately or only slightly checked, and one was apparently stimulated. Likewise, differences in response were observed when spores placed in water drops and on agar plates were exposed to diphenyl vapor. In the case of all the fungi so exposed, the developing hyphae were larger in diameter than normal and secondary branching was greatly increased. With some species of fungi there was a tendency to produce short, malformed or giant cells. In the presence of diphenyl the spores frequently became enlarged and distorted and often burst before germ tubes were developed to any appreciable extent. Death of cells did not occur except when they actually burst. Upon removal of diphenyl from cultures the organisms resumed growth and spore production, showing that the action of this chemical is fungistatic rather than fungicidal.

2. A new method of using diphenyl as a fungistatic agent in the control of citrus fruit decay was demonstrated. Unwrapped oranges placed in cuplike depressions in special paper-pulp trays impregnated with diphenyl developed little or no decay when packed in tight fiberboard boxes, whereas the controls developed considerable rot.

3. The control of rot in citrus fruit during transit, storage, and marketing through the use of diphenyl-treated wraps or trays is due to the inhibition of germination of contaminating surface spores, the retardation of growth of exposed hyphae already present on the

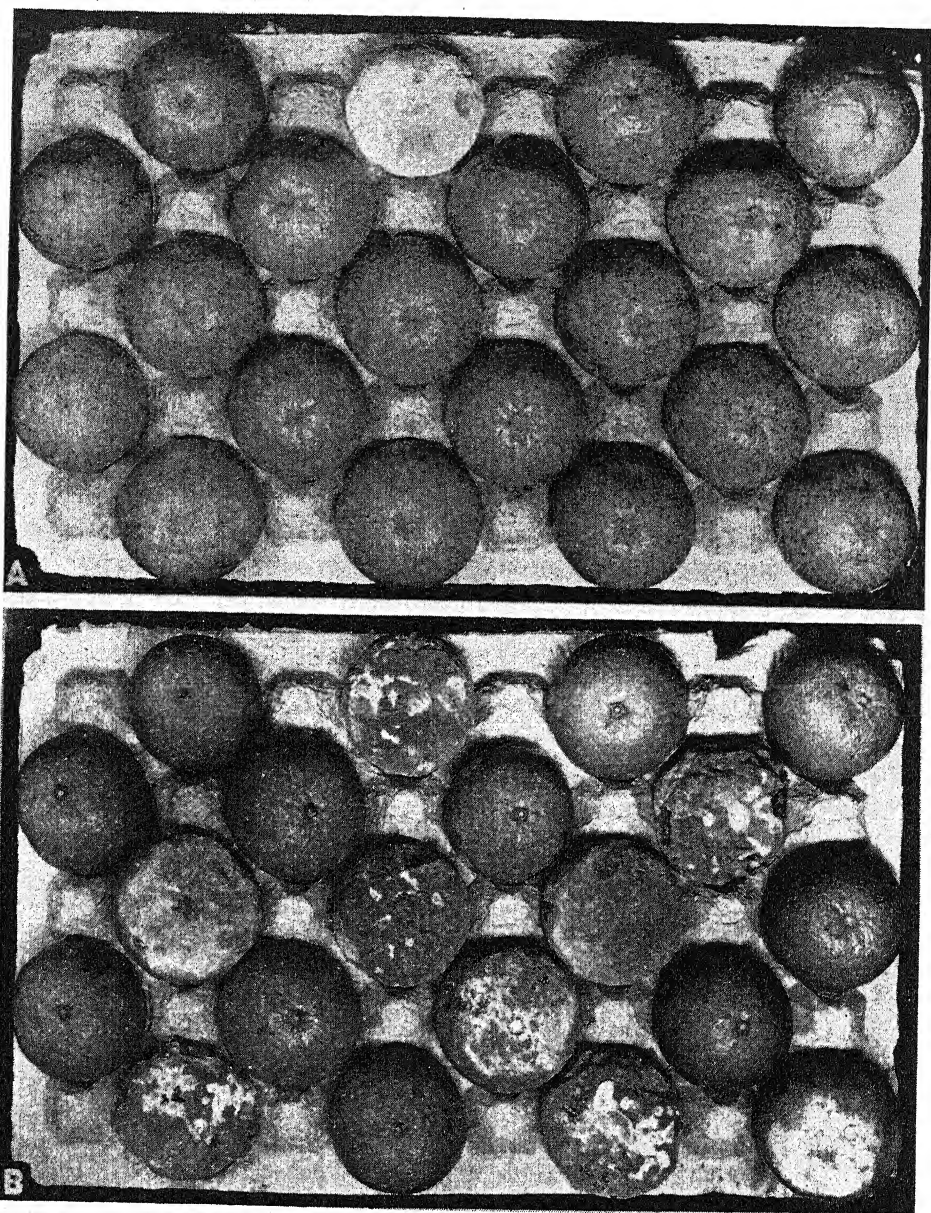


FIG. 3.—Effectiveness of diphenyl-treated trays in the control of green mold of oranges after 3 weeks at 72° F. and 40% relative humidity. *A*, diphenyl-treated tray; one decayed fruit out of 100 in test. Note white compact growth of mycelium and lack of spores. *B*, untreated tray, twenty-five decayed fruit out of 100 in test.

fruit, and the prevention of new spore formation that often is the source of infections during handling.

4. Diphenyl vapors should be effective in checking development of the following diseases of citrus fruit: blue mold rot (*Penicillium italicum*), *Botrytis* rot (*B. cinerea*), *Diplodia* stem-end rot (*D. natalensis*), green mold rot (*Penicillium digitatum*), and *Phomopsis* stem-end rot (*P. citri*). The failure of diphenyl to inhibit to any marked extent the mycelial

growth and spore germination of the causal organisms of *Alternaria* rot (*A. citri*), anthracnose (*Colletotrichum gloeosporioides*), brown rot (*Phytophthora citrophthora*), cottony rot (*Sclerotinia sclerotiorum*), and *Trichoderma* rot (*T. viride*) suggests that these diseases are not likely to be controlled satisfactorily by this chemical.

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EFFECT OF LIGHT INTENSITY AND NUTRIENT SUPPLY ON GROWTH AND PRODUCTION OF RUBBER AND SEEDS BY GUAYULE

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Introduction

Environmental factors, such as temperature, moisture, soil condition, and light, vary widely throughout the geographic range of guayule in the United States, and as a result there are variations in the amount of rubber produced by the plants in the different localities.

MILOVIDOV (5) studied light intensity as a factor in rubber production by certain plants. The formation of plant mass by *Asclepias cornuti* was decreased by 19.25%, and that of krym-saghyz by 43.2%, as the result of subjecting the

plants to light intensities that were 50% less than those which naturally prevailed. The amount of rubber produced was reduced to an even greater extent, indicating that light intensity was a relatively important factor in connection with rubber accumulation by these plants.

In comparing natural light conditions that prevail in different regions, it is necessary to rely on the relative number of hours of sunshine per year as an index rather than on actual intensity, since records are not available for the latter. It can be assumed, however, that the maximum intensities prevailing at relatively high elevations on a desert would exceed those recorded under more

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humid conditions at lower elevations.

With respect to light conditions that prevail at various locations in the guayule range, the average number of hours of sunshine per year at San Jose and Los Angeles (representing the central and southern coastal regions of California) was 6% less than that prevailing farther inland at Fresno, and 8% less than at El Paso, Texas (7)—the station nearest the natural guayule range at which hours of sunlight were recorded. On the basis of precipitation at Marathon and O2 Ranch, Texas, the percentage of sunshine that occurs on the natural guayule range in that state would appear to be even higher than at El Paso; but at Del Rio, Texas, where records were taken, 10% fewer hours of sunshine were recorded than for the coastal region of California.

Based on the percentage of total possible hours of sunlight, the yearly average at Beltsville, Maryland, where these experiments were undertaken, was lower by approximately 10-20% than that of most locations in the guayule range and approximately equal to that recorded at Del Rio. Furthermore, the relatively cold winters prevailing at Beltsville made it necessary to grow the plants in a greenhouse, where the light intensity was decreased still further by the glass during the period between October and February. Results presented therefore represent the behavior of plants grown in pots and subjected to a range of intensities, the mean of which was 10-30% less than that to which field plants are usually exposed. In the present experiments, plants grown in direct light at Beltsville attained, in a comparable period of time, a size equal to approximately one-third that of average irrigated field plants grown on the western guayule range and contained a slightly lower percentage of rubber (3).

Methods

Seedlings (variety 593) typical of those used for field plantings were obtained from the Alisal nursery of the Emergency Rubber Project near Salinas, California. They had grown from seeds during the previous summer season, and their branches had been trimmed to a length of 3-4 inches prior to transplanting. The seedlings were planted during the latter part of May in alluvial clay loam soil contained in 8-inch clay pots. They were then divided at random into eight groups, each containing ten uniform plants. Approximately 350 ml. of nutrient solution containing 35 milliequivalents of $\text{Ca}(\text{NO}_3)_2$ and 5 milliequivalents of KH_2PO_4 was applied to five pots of each group at intervals of from 3 to 6 weeks during the growing season, so as to maintain soil fertility. Soil in which the remaining plants grew was left untreated and decreased in fertility as the experiment progressed.

Two groups of ten plants each, including those growing in fertilized and also in unfertilized soil, were covered individually with frames over which was fastened a sufficient number of layers of tobacco cloth to reduce the light intensity to 75% of direct light, as measured by a Weston light meter. The intensity was reduced in a similar manner to 44% in the case of two other groups, to 24% of direct light in the case of two others, while the remaining two groups were left exposed to direct light. Treatments therefore consisted of four light intensities with two replications of each and two fertilizer treatments at each intensity level.

The frames used to support the cloth screen were approximately 3 feet wide, 6 feet long, and 2.5 feet high. The cloth screen with which these were covered extended to within approximately 6 inches

of ground level and slightly below the level of the rim of the pots, so as to allow for ventilation and to aid in equalizing the air temperature and humidity inside with that prevailing outside the tents. There was no appreciable difference in air temperature inside as compared with that outside the tents. Evaporation of water from the soil varied, depending on the light intensity. An attempt was made to maintain an optimum soil moisture content by the application of water to the soil when needed. No attempt was made to measure soil moisture content.

The seeds from the five plants of each replication of each treatment were collected during the growing season, which was divided into two parts—from June 1 to September 1 and from September 1 to October 1. After collecting, the seeds were cleansed and sorted into three sizes, with the aid of screens: those which passed a 10-mesh per inch screen, those which failed to pass through this but passed an 8-mesh screen, and those which failed to pass the 8-mesh screen. The seeds of each size gathered from the different treatments were then counted and subjected to germination tests.

In making these tests, four lots of 100 seeds were counted from each of the three samples. In cases where 400 seeds were not available, as many lots of 100 seeds as possible were obtained and the remainder used as one lot. Each lot of 100 seeds was treated and germinated separately. The 100 seeds were placed in shell vials and washed for 18 hours, three changes of 25 ml. of water being used in the washing. The seeds were then soaked for 2 hours in NaOCl (clorox) containing 1.5% available chlorine. Four milliliter of the hypochlorite was used for each lot of 100 seeds. The washing and soaking were carried out at room temperature. Following soaking in NaOCl,

the seeds were rinsed several times, then placed while still moist between germination blotters in the germinators, and allowed to germinate at alternating temperatures of 20° C. for 17 hours and 30° C. for 7 hours daily. The tests were continued for 21 days, the germinated seeds being counted and removed from the blotters every 3 days. Repeated trials have shown that these treatment and germination conditions insure the germination of between 98% and 100% of the mature seeds.

Germination tests of seeds of various sizes are recorded as the means of two replications of 400 seeds each, or 800 seeds in all. The average percentage germination produced by the plants in each treatment was calculated from the total number and percentage germination of the seeds of each size. These values are given as the mean germination percentage of the seeds for each treatment.

The germination data were analyzed statistically according to the method for percentage outlined by HAYES and IMMER (2). The term significant is used in this paper to mean that there were odds of 19:1 against such differences occurring by chance.

The experiment was transferred to a greenhouse in the latter part of October where the day temperature was 60°-70° F. and the night temperature 35°-45° F.

On February 16, 1944, all plants were harvested. The plants were defoliated, the green functioning leaves removed separately from those that were brown but still attached, and finally the stems and roots were ground together into coarse pieces. After the fresh weights of these samples were recorded, they were dried in a well-ventilated oven at 65° C. and the dry weights determined. Rubber and resin determinations were made by

means of the SPENCE and CALDWELL method (6).

As related to growth and rubber production, attention was directed toward the effect of light intensities on the anatomy of leaves and stems, and particularly the effect on the structure and proportions of bark, since it is the major rubber-storing part of the plant.

Stem segments were taken at the time of harvest from each of three plants grown in each of the four levels of light and the two fertilizer treatments. A second replication was similarly sampled, thus giving a total of six plants studied per treatment. One piece, approximately 1 cm. in length, was cut from the base of the main stem. In most cases this piece was taken immediately above the area of the cotyledonary plate, but in others, where low branches were present, the sample was slightly higher. Identification of exact areas was possible externally by the crowded leaf scars and internally by the appearance of medullary resin canals, which, according to LLOYD (4), do not develop in the hypocotyl but within the lower crowded internodes of the true stem. Sections of the stem were cut at 40μ with the aid of a sliding microtome equipped with a freezing attachment. The methods of preparing slides, obtaining measurements, and determining tissue areas have been described in an earlier paper (8). Cross-sectional areas of the pith, xylem, and bark, the last consisting of the tissues between the vascular cambium and cork cambium, were determined. Since the area of the bark functioning in rubber storage is reduced by the presence of fibers, cross-sectional areas of this tissue were also obtained. These data were subjected to an analysis of variance. In this paper the cross-sectional area of tissues was used as an

index of their volume per unit length of stem.

Leaves for histological study were also collected from plants of each treatment. Sections were cut at 6μ with the aid of a sliding microtome, stained with crystal violet, and mounted in glycerin jelly.

Results

GROWTH OF ROOTS, STEMS AND LEAVES.

—A reduction of 25% in light intensity significantly reduced the dry weight of stems and roots of plants grown in fertile soil, and more extreme reductions in intensity were associated with successive reductions in stem growth (table 1; fig. 1). In contrast, the growth of plants in infertile soil was limited, and the weight of their stems was not influenced by even a 56% decrease in light intensity. However, an extreme reduction of 76% in intensity significantly reduced the combined weight of stems and roots. The fresh weight of the entire plant decreased steadily with reduction in light intensity in the case of those grown in fertile soil. In contrast, the growth of plants grown in infertile soil was favored by a moderate amount of shading, while full sunlight or an extremely low light intensity (24%) decreased the amount of growth as measured by fresh weight of the entire plant.

Full light intensity did not favor the production of leaves by plants grown in infertile soil, and this factor largely accounted for the reduction in the weight of plants grown at the maximum intensity as compared with that of others which were shaded lightly.

The ability of plants to retain their leaves during the winter months varied in proportion to the intensity of light to which the plants were exposed. In Feb-

ruary, approximately two-thirds of the total dry weight of the leaves of plants grown in the highest intensity was represented by those that were green and functioning, while only one-third of the leaves of plants grown in a reduced intensity (24% level) had remained green and were functioning at that time.

SEED PRODUCTION.—Considering first the plants supplied with additional nutrient, the greatest number of seeds was

The greatest proportion of large-sized seeds was produced by plants grown with added nutrient and shaded lightly (75% of full intensity). Marked reduction in numbers of large-sized seeds resulted when the light intensity was reduced to less than 56% of unfiltered light. Withholding nutrients tended to increase the proportion of small and decrease the proportion of large seeds.

The largest seeds gave the highest per-

TABLE 1

WEIGHT OF LEAVES, ROOTS AND STEMS, AND ENTIRE POTTED PLANTS OF GUAYULE GROWN FOR 8 MONTHS UNDER DIFFERENT LIGHT INTENSITIES AND LEVELS OF NUTRITION. INTENSITY EXPRESSED AS PERCENTAGE OF DIRECT LIGHT. WEIGHTS REPRESENT AVERAGES OF TEN PLANTS EACH

PERCENTAGE INTENSITY	FRESH WT. WHOLE PLANT (GM.)		DRY WT. STEMS AND ROOTS (GM.)		DRY WT. TOTAL LEAVES (GM.)		TOTAL LEAVES AS PERCENTAGE OF WHOLE PLANT (DRY WT. BASIS)		GREEN LEAVES AS PERCENTAGE OF TOTAL LEAVES (DRY WT. BASIS)	
	Nutrient added									
	+	-	+	-	+	-	+	-	+	-
100.	66.7	36.7	37.2	19.5	15.0	3.5	28.7	15.4	67.7	65.5
75.	63.3	40.6	31.8	19.6	13.8	5.5	30.3	22.8	56.3	51.7
44.	55.0	39.0	27.5	19.3	11.7	5.2	29.7	21.3	54.0	50.8
24.	38.9	30.9	18.3	14.8	7.6	5.1	28.8	25.4	29.8	30.4

produced during June, July, and August by those plants grown under reduced light intensities (44%-75% of direct light). However, an extreme reduction in light intensity was unfavorable to the production of great numbers of seeds (table 2). During September, a greater number of seeds was produced by plants grown in direct light than by any others that were exposed to light of lower intensity. In the case of plants not supplied with additional nutrient, the greatest number of seeds was produced by those grown with a 56% reduction in light intensity during June, July, and August, and a 25% reduction during September.

centage of germination, the smallest seeds the lowest, and the medium-sized seeds an intermediate percentage. The germination of seeds gathered during September was significantly greater than that of seeds gathered during June, July, and August. While there were considerable differences in the number of seeds produced in the different treatments, there were no significant differences in the percentage germination (table 3).

ANATOMY.—Considering first the plants to which fertilizer was added, the stems showed a progressive decrease of the total cross-sectional areas in the three lower light intensities (table 4; fig.

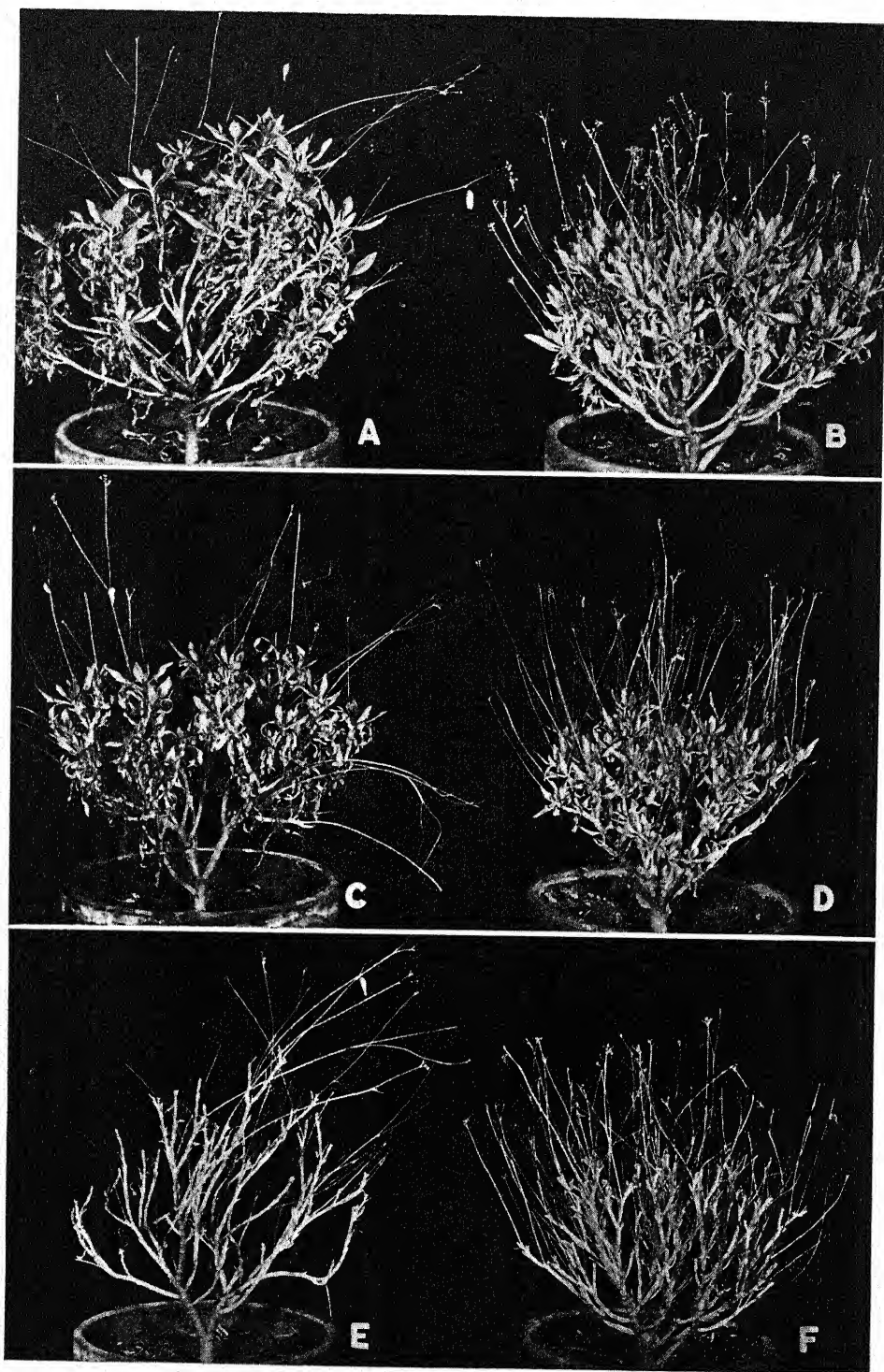


FIG. 1.—Guayule grown for 5 months during spring, summer, and fall under field conditions, followed by 4 months in greenhouse: *A*, in 24% of full light intensity and in soil to which nutrients were added; *B*, in soil to which nutrients were added and in full intensity; *C*, 24% of full intensity and without added nutrients; *D*, full intensity and without added nutrients; *E*, 24% of full intensity with nutrients added, plant after defoliation; *F*, full intensity and with nutrients added. Note effect of shading on number of branches, flower stalks, and dead leaves.

2). The stem area of those plants grown in direct light was not significantly different from that of other plants exposed to the 75% level. The areas of the different tissues composing the stem also decreased progressively in the three lower light intensities but did not differ significantly between the 75% level and

though the actual area of fiber was reduced by approximately 35%.

There was little difference in cell size or organization of parenchyma in the bark of plants grown with added nutrient and exposed to the different levels of light. Differences in extent of the tissue were apparently due to the number of

TABLE 2

NUMBER OF SEEDS OF DIFFERENT SIZES PRODUCED BY GUAYULE PLANTS GROWN UNDER VARIOUS LIGHT INTENSITIES AND NUTRIENT CONDITIONS. INTENSITY EXPRESSED AS PERCENTAGE OF DIRECT LIGHT

SEED SIZE	COLLECTED JUNE 1 TO SEPTEMBER 1, 1943				COLLECTED SEPTEMBER 1 TO OCTOBER 1, 1943			
	Percentage intensity							
	24	44	75	100	24	44	75	100
Large..... Medium..... Small..... Total....	Nutrient added							
	214	1484	1519	1376	89	259	370	295
	1729	2179	3221	2003	506	1523	2024	3291
	327	272	321	437	94	274	259	290
	2270	3935	5061	3816	689	2056	2653	3876
	Nutrient not added							
Large.....	169	553	865	626	35	82	148	29
Medium.....	1707	2745	2000	1580	219	689	856	511
Small.....	220	252	250	261	45	111	132	299
Total....	2096	3550	3115	2467	299	882	1136	839

direct light. There was a marked constancy of the proportions of each tissue, despite significant changes in over-all size of the stem at the different light intensities. The percentage area of bark showed no significant differences, although the actual area decreased by approximately 36%. Likewise, there were no significant differences in the percentage area of fiber in the bark of plants exposed to different light intensities, al-

cells produced in secondary growth, rather than to changes in the character of the cell. Large fiber cells in the bark were somewhat more numerous, and their walls somewhat thinner, in the shaded plants than in those exposed to unfiltered light.

In those plants grown without added nutrient, the actual cross-sectional area—both of the whole stem and of the various tissues—decreased significantly

TABLE 3

PERCENTAGE OF VIABLE SEED PRODUCED BY GUAYULE AT DIFFERENT SEASONS OF YEAR
AND UNDER VARIOUS LIGHT INTENSITY AND NUTRIENT LEVELS. INTENSITY
EXPRESSED AS PERCENTAGE OF THAT OF UNSCREENED LIGHT

SEED SIZE	COLLECTED JUNE 1 TO SEPTEMBER 1				COLLECTED SEPTEMBER 1 TO OCTOBER 1			
	Percentage intensity							
	24	44	75	100	24	44	75	100
Large..... Medium..... Small..... Mean....	Nutrient added							
	56.75	54.75	47.50	52.25	64.50	70.50	64.25	63.75
	43.00	30.75	40.00	34.25	44.25	51.50	49.50	50.00
	13.75	9.25	7.50	10.50	15.50	21.00	19.00	29.50
	39.42	39.66	41.00	38.82	42.75	49.75	48.68	49.26
	Nutrient not added							
	68.00	56.25	48.25	51.50	67.00	60.00	70.00	58.25
	40.75	29.25	31.50	28.75	48.75	51.50	53.25	48.00
	15.25	13.50	10.50	7.00	30.75	21.75	24.00	24.25
	40.09	32.21	34.36	31.99	47.30	48.84	51.80	40.55

TABLE 4

EFFECT OF DIFFERENT LEVELS OF LIGHT INTENSITY AND NUTRIENT ON AVERAGE CROSS-SECTIONAL
AREAS OF VARIOUS STEM TISSUES AND ON AVERAGE PERCENTAGE OF CROSS-SECTIONAL AREA OCCU-
PIED BY EACH TISSUE. INTENSITY EXPRESSED AS PERCENTAGE OF DIRECT LIGHT. AREA VALUES
REPRESENT AVERAGES OF SIX PLANTS EACH

PER- CENT- AGE IN- TEN- SITY	STEM		PITH				XYLEM				BARK				FIBERS			
	Total area (mm. ²)		Area (mm. ²)		Total stem area (%)		Area (mm. ²)		Total stem area (%)		Area (mm. ²)		Total stem area (%)		Area (mm. ²)		Bark area (%)	
	Nutrient added																	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
100. . .	112.6	89.5	2.4	2.6	2.1	2.9	49.9	37.3	43.6	41.6	60.3	49.7	54.3	55.5	13.3	12.6	22.5	25.5
75. . .	111.3	93.3	2.2	2.4	2.3	2.5	46.4	39.2	41.0	42.0	62.6	51.7	50.7	55.5	14.9	12.5	23.1	24.1
44. . .	96.0	82.1	2.4	2.2	2.5	2.6	40.0	33.2	41.6	40.4	53.7	46.0	55.9	57.0	11.9	10.4	22.2	22.1
24. . .	67.3	59.9	1.9	1.8	2.8	3.0	27.1	24.1	40.1	40.3	38.3	34.0	57.0	56.8	8.7	7.6	22.7	22.4

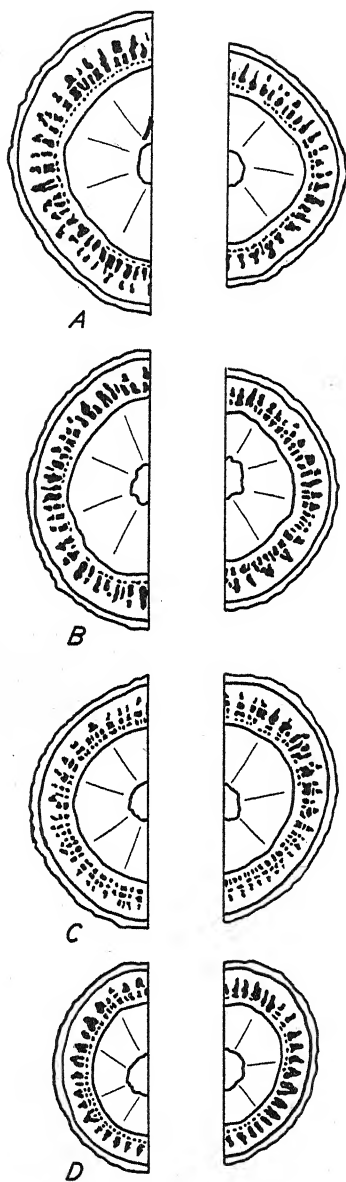


FIG. 2.—Tracings of projected images of stem cross-sections of guayule, those in left-hand column from plants grown in soil with nutrients added, right-hand column without added nutrients. *A*, plants grown in full light intensity; *B*, 75%; *C*, 44%; and *D*, 24% of full intensity. Areas of bark fiber darkened.

among the three lower levels of light, but there was no significant difference between the two highest levels. Greatest cross-sectional area of the component stem tissues occurred at the 75% light intensity level. The percentage areas of stem tissues of plants grown at the different light intensities and the different nutrient levels did not differ significantly.

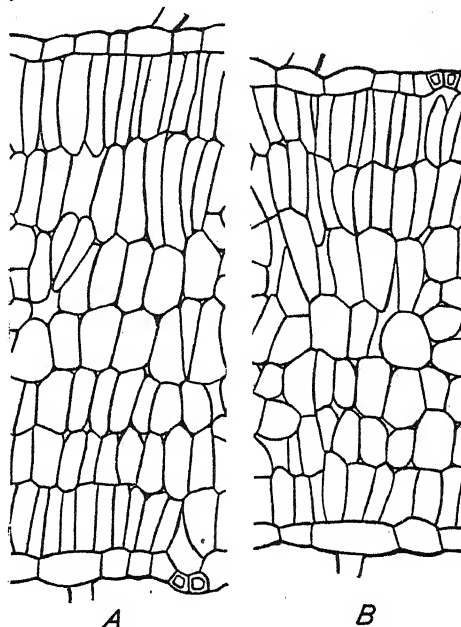


FIG. 3.—Cross-sections of leaves from guayule plants grown in soil to which nutrients were added: *A*, at full light intensity; *B*, at 24% of full intensity. Note only slight effect of shading.

Leaves of plants grown in the more fertile soil showed only a limited response to light intensity (fig. 3) with respect to amount and character of palisade and spongy parenchyma tissues. Leaf thickness decreased only slightly with shading. Leaves of plants grown at 75% of light intensity showed a 1% decrease in thickness compared with those of plants grown in direct light (6% and 12%, respectively, for plants exposed to 44% and 24% of full light intensity). GOUR-

LEY and NIGHTINGALE (1) found that in apple the leaves of plants in full light were 90% thicker than those of shaded plants, but in peach the sun leaves were only 20% thicker than shaded ones.

TABLE 5

PERCENTAGE AND ABSOLUTE AMOUNTS OF RUBBER IN STEMS AND ROOTS OF POTTED GUAYULE PLANTS GROWN FOR 9 MONTHS UNDER DIFFERENT LIGHT INTENSITIES AND NUTRIENT LEVELS. INTENSITY EXPRESSED AS PERCENTAGE OF DIRECT LIGHT. VALUES REPRESENT AVERAGES OF EIGHT PLANTS EACH

PERCENTAGE INTENSITY	RUBBER (%)		RUBBER PER PLANT (GM.)		POUNDS RUBBER PER ACRE (11000 PLANTS)	
	Nutrient added					
	+	-	+	-	+	-
100.....	5.13	3.71	1.91	0.69	46.4	16.8
75.....	3.88	3.38	1.23	0.66	29.9	16.0
44.....	3.43	3.39	0.94	0.64	22.8	15.5
24.....	1.92	2.60	0.34	0.37	8.3	9.0

Peach shed its leaves prematurely under shaded conditions. Likewise, the structure of guayule leaves was not changed appreciably by shading, and a marked increase in the proportion of dead leaves was noted in the case of heavily shaded plants.

RUBBER CONTENT.—Considering first the plants to which nutrient was added, a relatively high percentage of rubber accumulated in the stems of those grown in direct light (table 5). The stems of those grown in an intensity 25% less than that of direct light contained significantly less rubber on the absolute basis than did others grown in full light. More extreme shading of the plants was accompanied by a corresponding decrease in percentage stored. In contrast, both

the percentage and the absolute amount of rubber in stems of plants grown without added nutrient were relatively low, and neither was significantly reduced by shading except at the lowest light level (24% of unscreened light).

RESIN CONTENT.—The percentage of resin in stems and roots of plants exposed to full light intensity was not influenced by the addition of nutrients (table 6), and the percentage in similar parts of shaded plants was greater in low nutrient plants than in those to which nutrients were added. Owing to their smaller size, however, the absolute amount of resin in plants grown without added nutrient was considerably less than that found in those grown in the more fertile soil. Moderate shading (75% of full intensity) resulted in a 25% de-

TABLE 6

PERCENTAGE AND ABSOLUTE AMOUNTS OF RESIN IN STEMS AND ROOTS OF GUAYULE GROWN FOR 9 MONTHS UNDER DIFFERENT LIGHT INTENSITIES AND NUTRIENT LEVELS. INTENSITY EXPRESSED AS PERCENTAGE OF DIRECT LIGHT. RESIN VALUES REPRESENT AVERAGES OF TEN PLANTS EACH

PERCENTAGE INTENSITY	RESIN (%)		RESIN PER PLANT (GM.)	
	Nutrient added			
	+	-	+	-
100.....	5.0	5.0	1.86	0.98
75.....	4.4	4.9	1.40	0.96
44.....	4.3	4.6	1.18	0.89
24.....	3.2	3.6	0.59	0.53

crease in the resin output of plants to which additional nutrients were supplied, in contrast to a 2% reduction in the resin output of those to which nutrients were not added.

Discussion

When grown at a relatively high nutrient level, plants of the present experiment were extremely sensitive to reduction in light intensity with respect to their total output of rubber. The most moderate shading reduced by 36% the absolute amount of rubber produced. There was an approximate linear relationship between the amount of rubber stored per plant and the intensity of light to which the plants were exposed (fig. 4). Even at the highest intensity used, light was a major limiting factor with respect to the total amount of rubber accumulated by plants grown in the more fertile soil. Shading was also associated with a decrease in concentration of rubber in the roots and stem tissues, and with a decrease in the over-all growth of the plants, as reflected in the weight of the leaves, roots and stem. Decrease in the stem diameter and cross-sectional areas of the various tissues occurred in the three lower light intensities, but there was no significant difference between the cross-sectional areas of those exposed to 75% and 100% of the intensity of direct light. The decrease in stem weight associated with moderate shading (75% level) may be accounted for on the basis of a decrease in the number of branches, an effect which became more striking as the light intensity was reduced, and which was most clearly evident in plants subjected to the lowest intensity (fig. 1E, F).

In contrast, those plants grown at a low nutrient level were relatively insensitive to variations in light intensity. Their vegetative growth and rubber content were significantly less than that of plants grown with added nutrient, irrespective of the intensity in which they were grown. Prolonged inhibition of

vegetative growth, the result of maintaining the plants on a low nutrient level, was not associated with accumulation of carbon in the form of rubber, even in the case of plants exposed to direct light. Apparently the process of rubber synthesis, as well as the synthesis of other materials by these plants, was limited by an inadequate inorganic nutrient supply. Synthesis of resin, on the other hand, was not limited by an inadequate nu-

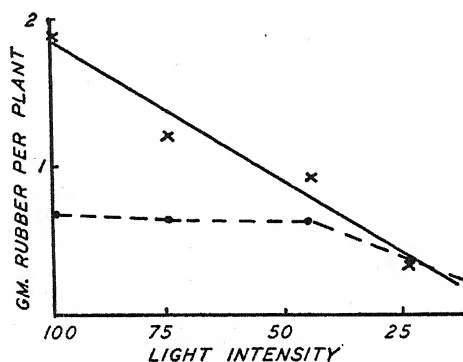


FIG. 4.—Effect of shading on amount of rubber produced by guayule grown at different nutrient levels and light intensities. Intensity expressed as percentage of that of unscreened light at Beltsville. Solid line represents plants to which additional nutrient was supplied; broken line, those grown without added nutrient.

trient supply, since the percentage of resin in the stems and roots of the smaller low nutrient plants was equal to or greater than that recorded for the larger plants to which nutrients were supplied.

Reduction in light intensity was associated with marked decrease in the percentage of leaves which remained green and functioning during the winter. The limited anatomical response of leaves to shading may have in part accounted for the fact that heavily shaded plants lost most of their green leaves during the winter season. This factor may have reduced to some extent the rubber-producing capacity of the shaded plants. The

tendency for moderately shaded plants grown in the more fertile soil to produce a relatively great number of seeds during the summer months may also have been a factor in limiting the amount of rubber produced by these plants during the following dormant season. The relative proportions of tissues composing the stem show no relationship to changes in nutrient treatment, variation in light intensity, or rubber concentration. The amount of rubber produced by shaded plants was therefore not limited by available rubber-storing area. It is evident that the limited rubber output of shaded plants grown with added nutrient was associated with (a) a decrease in the total synthesis of solid matter by the plant, (b) an increase in seed production under conditions of moderate shading, and (c) a decrease in the number of functional leaves during the winter rubber-storing season.

Summary

1. Potted plants of guayule were grown for a period of 5 months during the spring, summer, and fall in natural light of full intensity, and other plants were subjected to lower intensities obtained by means of cloth filters. Half the total number of plants were supplied with nutrient at intervals to maintain soil fertility, while the remainder were grown in relatively infertile soil. During the winter the experiment was transferred to a greenhouse and continued under controlled temperature conditions.

2. A 25% reduction in light intensity was associated with a 36% reduction in the amount of rubber produced by plants grown in relatively fertile soil. Further reduction of light was associated with a corresponding decrease in the percentage and absolute amount of rubber stored. The percentage and absolute

amounts of rubber produced by plants grown in infertile soil were relatively low and not greatly affected by shading.

3. A reduction of 25% in light intensity reduced the dry weight of stems and roots of plants grown in fertile soil by 14.5%; and more extreme reduction in light further retarded vegetative growth. Of plants grown in infertile soil, the greatest leaf weight was produced by those exposed to 75% of full sunlight, but moderate shading had no appreciable effect on the weight of stem and roots.

4. Two-thirds of the leaves of plants grown in direct light remained green throughout the winter, but only one-third of those on deeply shaded plants remained green and functioning.

5. Reduced light intensity (44%-75% of unscreened light) favored seed production during June, July, and August. Shading was less effective in stimulating seed production during September. Decrease in light intensity was associated with decrease in the proportion of large seeds produced. Withholding inorganic nutrients was associated with an increase in the proportion of small and a decrease in the proportion of large seeds. Reduction in light intensity was not associated with an appreciable change in seed quality.

6. The cross-sectional area of stems was not significantly affected by a 25% reduction in intensity; but further reduction was associated with marked decrease in stem thickness, cross-sectional area of pith, xylem, bark, and fiber tissue in the bark.

7. Percentage areas of the various stem tissues were essentially constant both in the different light intensities and with the two fertilizer treatments. The percentage of bark was not correlated with changes in percentage of rubber.

8. Under full light intensity, the per-

centage of resin in plants grown with a limited nutrient supply was equal to that of plants to which nutrients were supplied; and with shading those subjected to a limited nutrient supply contained a higher percentage of resin than did those afforded nutrients.

All rubber and resin determinations were made by Mr. R. L. HOLMES and statistical analyses by Mrs. MARY W. SHANOR.

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WINTER HARDINESS IN GUAYULE

JOHN W. MITCHELL¹

Introduction

Plants of guayule have withstood winter temperatures of 5° F. at Marathon, Texas, and 10° F. at Tuscon, Arizona (3). A temperature of -5° F. has been recorded at El Paso, Texas, near the geographic range of guayule. At Fort Stockton, Texas, within the natural range and near its northern limit, a minimum of -7° F. has been recorded. From this it appears that this plant is able to withstand relatively low temperatures, at least for a short time, in some localities in the United States.

On the other hand, Russian investigators have reported (2, 4) that the varie-

ties with which they worked did not live when subjected to temperatures of 5°-14° F. unless the plants were given "special attention." By subjecting some of them to a special pre-wintering treatment, the plants were made to survive -4° F. It was concluded that the only means of increasing the resistance to low temperatures was by cross-breeding it with resistant types such as Origan, a variety of guayule selected for use in Russia.

BENEDICT (1) observed that potted field-grown transplants, variety 593, were killed at approximately 20° F., while yet in an unhardened condition. Even after hardening for 2 months at temperatures close to 20°, the plants were not able to withstand 15° F. Plants grown in soil with a low moisture content were no more resistant. A few young

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seedlings survived 15° overnight, but were killed by exposure to 10°. BENE-DICT concludes that one-year-old plants of guayule of the variety used cannot be hardened sufficiently to withstand temperatures of 10° F. or below without some injury; but he suggests that there may be other cultivated varieties, or some wild plants, which are less susceptible to frost injury than those tested.

This paper reports experiments undertaken for the purpose of comparing different ages and varieties of guayule plants with respect to their ability to withstand low temperatures, and in addition to observe the effect of frost injury on their rubber content.

Methods

Seeds of several selections or varieties were planted under comparable conditions on the same date. These included nos. 406, 593, A-5058, and A-4278. The young plants were later potted, and part of them were kept in a greenhouse during the summer and fall so that they would produce relatively succulent growth, while the remainder were kept outdoors. All four varieties were subjected to the same conditions, irrespective of the locations where they were grown. As the outside plants hardened off during the fall and winter, repeated tests were made with both greenhouse and outside plants to compare their resistance to low temperature injury. In some instances the rubber content of frost-injured plants was compared with that of uninjured plants. In addition, similar experiments were carried out with more mature potted and field-grown plants.

In general, three methods were used to test the hardiness of guayule: (a) potted plants were subjected to controlled temperatures for known periods of time;

(b) potted plants were subjected to prevailing outdoor temperatures; and (c) unprotected and protected field plantings were subjected to prevailing outdoor temperatures. Six plants of a kind were used for each test, and the experiments were generally repeated several times.

Results

RESISTANCE OF SUCCULENT COMPARED WITH THAT OF NONSUCCULENT PLANTS.—Seedling plants of varieties 406, 593, A-4278, and A-5058 produced very succulent growth under greenhouse conditions. In contrast, plants of the same varieties and age grown outdoors produced a sturdy woody type of growth. Neither the greenhouse nor the outside plants had been subjected to temperatures which would appreciably increase their frost resistance prior to the date of these early experiments.

Tolerance to low temperatures was determined by subjecting the plants to controlled temperatures for a period of 18 hours. At the beginning of the experiments the plants experienced a sudden drop in temperature to approximately 50° F., since they were moved during the afternoon from prevailing greenhouse or outside temperatures directly to the desired controlled temperature. After cold treatment, they were placed at 50°–60° F. in diffused light until the soil reached air temperature again. Results of this and a similar experiment using only woody plants are given in tables 1 and 2. There were no important varietal differences in the resistance of unhardened seedlings to prolonged cold treatments. Succulent plants were severely injured at 24°, while woody plants were injured approximately the same amount by exposure to 20° F.

In another experiment, the temperature of the air surrounding unhardened

but woody outdoor seedlings was gradually decreased from 63° at 10 A.M. to 24° F. at 4 P.M. and held at 24° F. for a period of 5 hours. During this interval the soil temperature decreased from approxi-

TABLE 1

RESISTANCE OF SUCCULENT AS COMPARED WITH LESS SUCCULENT GUAYULE SEEDLINGS TO LOW TEMPERATURES. PLANTS EXPOSED TO CONTROLLED TEMPERATURES FOR 18 HOURS. RESPONSES EXPRESSED AS DEGREE OF INJURY TO LEAVES AND BRANCHES BASED ON SIX PLANTS FOR EACH TREATMENT. OBSERVATIONS MADE DURING 6-WEEK PERIOD FOLLOWING TREATMENT

VARIETY	TEMPERATURE (° F.)		
	31°	28°	24°
Succulent greenhouse plants			
A-4278.....	None	Slight*	Severe
406.....	None	Slight	Severe
A-5058.....	None	Slight	Severe
593.....	None	Slight	Severe
Woody outdoor plants			
A-4278.....	None	None	None
406.....	None	None	None
A-5058.....	None	None	None
593.....	None	None	None

* Slight: Leaf injury; branches not injured sufficiently to result in appreciable set-back in growth. Moderate: Many leaves killed and some branches injured for 1-3 inches back from tip. Severe: Most leaves killed; branches injured back to main stem, so that plants would suffer severe set-back even though they survive; often associated with root injury.

mately 60° to 23°. The air temperature was gradually increased to 70°, and finally the plants were returned to the greenhouse. Examinations 2 months later showed that all plants had been killed. It was concluded that when limited to a relatively short period of time, the rate of cooling—preceding exposure to low temperatures—did not appreciably alter the resistance of the plant to injury.

EFFECT OF DURATION OF COLD TREATMENT ON SEEDLING PLANTS.—Potted seedling plants, variety 593, grown outdoors, were subjected in groups of six to 24° for 4, 8, 12, and 16 hours, respectively. The minimum soil temperature in the case of each respective group was 32°, 30°, 30°, and 27° F. Exposure to an air temperature of 24° for 4 hours and to a minimum soil temperature of 32° resulted in only slight leaf injury. Exposure to an air temperature of 24° for 16 hours and a

TABLE 2

RESISTANCE OF TOPS AND ROOTS OF WOODY OUTDOOR GUAYULE SEEDLINGS TO LOW TEMPERATURES. RESPONSES EXPRESSED AS DEGREE OF INJURY BASED ON SIX PLANTS FOR EACH TREATMENT. OBSERVATIONS MADE DURING 6-WEEK PERIOD FOLLOWING TREATMENT

VARIETY	TEMPERATURE (° F.)					
	27°		20°		16°	
	Tops	Roots	Tops	Roots	Tops	Roots
593.....	None	None	Severe	Severe	Killed	Killed
A-4278..	None	None	Severe	Severe	Killed	Killed
406.....	None	None	Severe	Severe	Killed	Killed
A-5058..	None	None	Severe	Mod- erate	Killed	Killed

minimum soil temperature of 27° resulted in leaf injury, which did not appreciably inhibit growth of the seedlings. In another experiment, seedlings of variety 406 were subjected to a temperature of 22° for 4, 8, 12, and 16 hours (table 3).

It is concluded that unhardened seedlings might be expected to withstand long exposures to a temperature of 25°, provided the soil temperature did not range below 27° F. Exposure to temperatures as low as 22° for 8-12 hours or more, however, resulted in severe injury to unhardened seedlings.

FROST RESISTANCE OF MORE MATURE PLANTS.—In preliminary experiments, unhardened potted plants 15-18 months old withstood overnight exposure to a

temperature of 20° F. To study the effect of lower temperatures, hardened plants of the same age, grown outdoors, and also unhardened greenhouse plants, were exposed to controlled temperatures of 18°, 14°, and 10° for a period of 18 hours.

TABLE 3

FROST INJURY OF GUAYULE SEEDLINGS AS RELATED TO DURATION OF EXPOSURE TO LOW TEMPERATURES. RESPONSES EXPRESSED AS DEGREE OF INJURY BASED ON SIX UNHARDENED OUTDOOR SEEDLINGS FOR EACH TREATMENT. TREATMENTS APPLIED OCTOBER 16

EXPOSURE TIME (HOURS)	TEMPERATURE (° F.)		PLANT RESPONSE DECEMBER 16
	Air	Soil minimum	
4.....	22	32	Only slight leaf injury
8.....	22	30	Only slight leaf injury
12.....	22	28	Severe injury
16.....	22	25	Killed

The outdoor plants had previously been exposed to moderately cool nights for 3 weeks, during which time the minimum night temperature was 35° and the average minimum night temperature 46° F. (table 4). This experiment was repeated three times with similar results, which illustrated that unhardened potted plants 15-18 months old were no more resistant to frost injury than were young seedlings 6-8 months old. The experiment demonstrated, however, that the upper stems and branches of the older plants could be hardened so that they would withstand exposure to 10° for a period of 18 hours with only slight injury. In contrast to the above-ground parts, the roots of these hardened plants were injured so severely, even at an air temperature of 18°, that death resulted.

Results of a subsequent experiment using potted plants of different varieties

and ages also demonstrated that the roots of guayule were relatively sensitive to low soil temperatures. Seedling plants 8 months old, including varieties 593, 406, A-4278, and A-5058, were grown at low temperatures in a coldframe during the fall to increase their resistance to frost injury. More mature plants, variety 593, 15-18 months old, were also grown under the same conditions. On December 21, these hardened plants, plus some unhardened greenhouse ones (variety 593, 15-18 months old), were exposed to prevailing outside temperatures. On January 3 all plants were transferred to the greenhouse and examined for frost injury. The stems of the more mature plants became acclimated to low temperatures and were thus able to withstand exposure to 4° F. (table 5), but the roots were severely injured under these

TABLE 4

FROST INJURY OF SUCCULENT GREENHOUSE GUAYULE PLANTS 15-18 MONTHS OLD, COMPARED WITH THAT OF MORE WOODY PLANTS OF SAME AGE GROWN OUTDOORS. RESPONSES EXPRESSED AS DEGREE OF INJURY TO THAT PART OF PLANT ABOVE SOIL LEVEL. RESULTS BASED ON SIX PLANTS FOR EACH TREATMENT. DURATION OF EXPOSURE 18 HOURS

TEMPERATURE (° F.)	ONE WEEK AFTER TREATMENT		TWO MONTHS AFTER TREATMENT	
	Greenhouse	Outside	Greenhouse	Outside
18.....	Severe	Slight	Killed	Killed
14.....	Very severe	Slight	Killed	Killed
10.....	Very severe	Slight	Killed	Killed

conditions. Stems of the younger plants failed to become acclimated sufficiently to withstand an exposure to the minimum prevailing temperatures.

EFFECT OF DURATION OF COLD TREATMENT ON MORE MATURE PLANTS.—In pre-

TABLE 5

FROST RESISTANCE OF GUAYULE SEEDLINGS AS COMPARED WITH MORE MATURE PLANTS. RESULTS EXPRESSED AS DEGREE OF INJURY BASED ON SIX PLANTS PER TREATMENT

VARIETY AND AGE OF PLANTS	MINIMUM TEMPERATURE (° F.)				OBSERVATIONS
	Six weeks preceding experiment		During test		
	Range	Mean	Range	Mean	
406, 8 months.	21-55	35	4-32*	18	Killed
593, 8 months.	21-55	35	4-32	18	Killed
A-4278, 8 months.	21-55	35	4-32	18	Killed
A-5058, 8 months.	21-55	35	4-32	18	Killed
593-15, 18 months (out- side).....	21-55	35	4-32	18	Roots killed; tops not injured
593-15, 18 months (green- house).....	36-60	43	4-32	18	Killed

* Minimum temperature of 4° prevailed on December 24; on all other days it remained between 8° and 32° F.

liminary experiments it was observed that unhardened greenhouse plants 15-18 months old were killed by exposure to 0° F., even though the duration of exposure was only 3 hours. Additional experiments were made, using plants 15-18 months old of varieties 593 and A-5058. These plants had grown in pots under comparable conditions outdoors during the fall at minimum night temperatures of 32°-58° F. An average of 39° F. prevailed for the 3 weeks preceding the experiment.

On November 8 the plants were subjected to controlled temperature conditions as indicated in table 6. Varietal difference was observed with respect to frost resistance, since hardened plants, variety 593, were killed by exposure to an air temperature of -5° for a period of only 3 hours, while plants of variety A-5058 survived with only slight root injury (table 7). In general, the results indicate that even the most hardy of the potted plants were unable to withstand

exposure to an air temperature of -5° and a soil temperature of 16° F. for longer than a few hours.

TABLE 6

TEMPERATURE CONDITIONS UNDER WHICH FROST RESISTANCE OF PRE-HARDENED GUAYULE PLANTS 15-18 MONTHS OLD WERE TESTED

TIME	HOURS OF TREATMENT	TEMPERATURE (° F.)	
		Air	Soil
Nov. 8: 10 A.M.....	0	- 5
1 P.M.....	3	- 5	16
4 P.M.....	6	- 5	5
7 P.M.....	9	- 5	- 4
7 P.M. to 8 A.M.....		35	32
Nov. 9: 11 A.M.....		40-50	32
1 P.M.....		50-60	32
3 P.M.....		50-60	54
4 P.M.....		50-60	59

WINTER HARDINESS OF FIELD-GROWN PLANTS.—Some plants of variety 593, 8-10 months old, were left unprotected in the field during the fall and part of the

winter, while others of the same age and variety were completely covered with pine needles to maintain a relatively high temperature in the air and soil immediately surrounding the plants. Other

TABLE 7

VARIETAL DIFFERENCE IN RESPONSE OF GUAYULE PLANTS TO TEMPERATURE CONDITIONS LISTED IN TABLE 6. RESPONSES EXPRESSED AS DEGREE OF INJURY BASED ON GROUPS OF SIX PLANTS PER TREATMENT

VARIETY	DURATION OF EXPOSURE (HOURS)		
	3	6	9
593.....	Killed	Killed	Killed
A-5058...	Only fibrous roots injured; stems uninjured	Killed	Killed

similar plants were covered with a wooden box to eliminate light. All plants remained under these conditions for 3 weeks previous to a relatively cold period of 12 days. A week later, the plants were transferred to a greenhouse and examined to determine the extent and location of injury.

It was possible to harden the above-ground portions of field-grown plants (table 8). In such condition the stems and small branches of field plants withstood a minimum temperature of 6° and an extended cold period during which the minimum temperature declined to 15° or below for a period of 12 consecutive days. The roots of field plants failed to survive these conditions, however, even though the minimum soil temperatures were not below 26.6° F. The absence of light during a period of approximately 5 weeks did not appreciably change the resistance of the plants to frost injury.

The present results indicate that guayule can readily be protected against temperatures near 0° F., and probably subzero weather, by means of a mulch. Such protection may be of interest in connection with experimental plantings.

EFFECT OF INJURY ON RUBBER CONTENT.—Plants of different ages were treated. One group was kept in a greenhouse having a minimum temperature of 40° and a usual daily range of 45°–65° F. Another group was kept in a coldframe

TABLE 8

RESPONSE OF FIELD-GROWN GUAYULE PLANTS TO LOW TEMPERATURE. DEGREE OF INJURY BASED ON GROUPS OF FIVE PLANTS PER TREATMENT

CONDITION	MINIMUM TEMPERATURE (° F.) FOR 3 WEEKS PRECEDING LOW PERIOD	LOW TEMPERATURE PERIOD (DEC. 14-25)			INJURY OBSERVED JAN. 3
		Minimum air temperature		Minimum soil temperature at depth 3"	
		Range	Mean		
Unprotected....	15-38	6-15	11	26.6	Severe root injury; no apparent top injury
In darkness.....	21-42	10-19	14	Severe root injury; no apparent top injury
Covered with mulch.....	21-53	18-27	22	35.6	No apparent root or top injury

at temperatures generally ranging between 25° and 45°, with a minimum of 21°, which caused no apparent injury. The third group was grown in a cold-frame at approximately the same temperatures, except that the plants were exposed to a minimum of 6° during one night. These were severely frost-injured. Subsequent analyses indicated that the rubber content of the frost-injured plants was not significantly different from that of those uninjured (table 9).

TABLE 9

EFFECT OF FROST INJURY ON RUBBER CONTENT OF GUAYULE. FIGURES REPRESENT PERCENTAGE RUBBER IN DRIED STEMS OF SEEDLING PLANTS ASSAYED WITHIN 2 MONTHS AFTER TREATMENT. TEMPERATURES REPRESENT MINIMUM TO WHICH EACH GROUP WAS EXPOSED

PLANT NO.	PLANTS 8 MONTHS OLD (° F.)			PLANTS 15 MONTHS OLD (° F.)		
	40°	21°	6°*	40°	21°	6°*
1.....	2.4	3.6	2.0	4.8	3.9	3.7
2.....	2.4	2.2	2.5	4.0	4.3	3.5
3.....	3.8	3.1	2.2	4.8	2.6	3.2
4.....	2.4	2.8	2.6	1.8	3.5	5.4
5.....	2.1	2.7	4.7	3.8	4.5	2.0
Average...	2.6	2.9	2.8	3.8	3.8	3.6

* Plants exhibited symptoms of very severe frost injury.

In a similar experiment two groups of five plants each were grown under field conditions. Plants of one group were covered with a mulch, while the others were left unprotected. The former, subjected to a minimum of 20° F., showed no evidence of frost injury. The latter were exposed to a minimum of 6° F., which resulted in the death of root and stem tissues of each plant up to approximately 1 inch above the ground level. The leaves were also killed, but the upper portions of the stem and main branches were uninjured. Rubber analy-

ses made within 2 months after treatment showed an average content of 3.3% for the protected and 3.7% for the unprotected plants.

In another experiment, sixteen uniform unhardened plants grown in a greenhouse with a daily range of approximately 45°-65° and a minimum of 40° were divided into two equal groups. One was subjected to 18° F. for a period of 18 hours, the other was kept at room temperature. Roots, leaves, and small branches of the cold-treated plants showed evidence of frost injury. Analyses of the individual plants made approximately 1 month later showed no significant difference between the percentage of rubber in the injured as compared with that of the uninjured plants, the means being 4.4 and 4.6, respectively.

Conclusions

On the basis of the present experiments, seedlings—and also more mature plants of the type generally grown for rubber—did not withstand temperatures of 15°-20° F. for more than a few hours at a time without injury, unless they had previously been acclimated to low temperatures. The reason for this relatively high critical temperature seems to lie mainly in the fact that roots of guayule are subject to injury when exposed to a soil temperature of 26°-28° F. for a period of only 8-10 hours. Like many other kinds of woody plants, the stems and leaves of the more nearly mature ones can be readily acclimated to low air temperatures by subjecting them to a hardening period of several weeks. In a winter-hardened condition, the stems of guayule transplants withstood repeated and prolonged exposures to temperatures ranging 5°-10° above zero, and in plants of variety A-5058, the stems survived an

exposure of 3 hours at 5° below zero with no apparent injury. There was no evidence that the resistance of roots to low temperature increased as the result of pre-hardening. However, most soil temperatures used in these tests were relatively low and caused severe injury. It is possible that slight differences in the resistance of roots to low soil temperatures would have been apparent had more moderate soil temperatures been used.

Sharp differences were observed when the frost resistance of two varieties of older plants were compared, and some variation was noted in the resistance of individual plants within the variety A-5058. It is therefore apparent that field plants may vary in their ability to withstand exposure to low temperatures and that some variation in this respect might be expected among the individuals in some of the known varieties or selections. On this basis, a selection and cross-breeding program would offer a promising means of increasing the average winter hardiness of guayule plants.

Summary

1. Woody outdoor seedlings of guayule not yet unacclimated to low temperatures survived only slightly lower temperatures than did very succulent greenhouse plants of the same age.
2. The rate of cooling, preceding an extended exposure to low temperatures, did not appreciably alter the resistance of the plant to frost injury.
3. Unhardened seedlings of four varie-

ties withstood prolonged exposure to a temperature of 25° but were severely injured as the result of an 8-10-hour exposure to 22° F.

4. In most instances, the roots were severely injured by prolonged exposure to soil temperatures of 26°-28° F. As an exception, roots of plants of selection A-5058 survived a short exposure (less than 3 hours) to a soil temperature of 16° F.

5. Stems and leaves of transplants were readily acclimated by means of low temperature hardening treatments, so that they withstood repeated exposure to temperatures ranging 5°-10° above zero. Stems of hardened plants of selection A-5058 withstood a 3-hour exposure to a temperature of 5° below zero. The roots of these plants were slightly injured but the plants survived.

6. Based on a limited study of four selections, plants of A-5058 were most resistant to frost injury.

7. Experimental field plantings can readily be protected from temperatures as low as 4° F., and probably lower, by means of an adequate mulch.

8. The rubber content of plants severely injured by frost was not significantly different from that of comparable uninjured plants during a period of 1-2 months after treatment.

All rubber determinations were made by Mr. R. L. HOLMES.

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STUDIES OF DEVELOPMENT IN LARKSPUR. I. FORM SEQUENCE IN THE FIRST TEN MATURE LEAVES

SPENCER W. BROWN

Introduction

Homologous organs formed in series offer advantages to the student of growth and developmental relationships in plants. Since individual organs, as successive leaves borne by a growing stem, may be inspected or later removed without injury to the organism as a whole, only a small number of plants need be grown to secure a relatively great number of measurements. Thus a single organ complex can be analyzed through all growth phases without sacrificing material. In addition, definite intervals of physiological time are available, especially useful when time is to be cancelled out, as in studies of allometry.

WHALEY (6) found that the size of organ produced could be directly correlated with the size of the apical meristem at any time during development. From the four or five measurements he gave for the entire active growth period, it is impossible to tell whether the ontogeny of the apical meristem follows a parabolic or a sigmoid developmental pattern, but the latter seems more likely. ABBE, RANDOLPH, and EINSET (1) were able to show that increase in width of corn leaves numbers 6 through 12 can be attributed to a corresponding increase in size of the growing point. Although detailed data are given by these writers to show the parallel growth patterns of successive leaves, analysis is lacking of the changes in size as a function of time of either the growing points or the mature leaves. It was obviously necessary to establish first a relationship between apex and developmental pattern which would then allow changes in adult leaves to be interpreted, at least in part, as reflections of underlying changes in growing points.

The present report is intended to pro-

vide preliminary information on factors regulating leaf development in larkspur (*Delphinium ajacis* L.) by an analysis of changes in the first ten mature leaves of several characters—number of points, length, area, and area/length.

MATERIAL AND METHODS.—Cultivated double-flowering larkspurs were grown in the greenhouse at Athens, Georgia, during the winter 1943-44, under approximately uniform conditions. The seedlings were transplanted to 2-inch pots at about the second-leaf stage, and at about the five-leaf stage were carefully reset in 5-inch pots. Although definitely not isogenic, the material seemed fairly uniform in regard to the characters employed in the present study.

Counts of the number of points per leaf (fig. 1) were made shortly after the young leaves had grown out completely from the terminal bud. Of course, the number of points remained the same during later development of individual leaves. Leaves were later removed from the plants for measurement when they began to turn a lighter shade of green, or yellowish at the margins, and it was apparent that their usefulness to the plant was probably finished. By the time the second or third leaf was removed, the tenth seemed already to be completely matured. The leaves were pressed lightly for a few hours, and then silhouettes obtained on photosensitive paper as long as the paper was available. Later it became necessary to use the leaves themselves in projection. Tracings were made of projections at five or ten diameters; from these, area and length measurements were made with planimeter and steel rule, respectively. Length measurements are of the blade only and do not include the petiole (fig. 1, leaf 1).

After the point number counts were made, some of the leaves were injured, either by handling or by insects. If more than one or two leaves were injured, the plant was completely eliminated from all but the point counts which had been made previously. The point counts plotted against length and area are from the same plants on which the length and area measurements were made. In all cases where there was a variation in total number from leaf to leaf (for example, $N=26-28$), the same group of plants (here twenty-eight) was used throughout.

Developmental sequences

The first four to six larkspur leaves are borne in a rosette, later leaves coming above successively longer and longer internodes. The longest internodes occur between the twelfth or thirteenth leaf and the inflorescence. Apparently there is a decided relationship between length of petiole and position at which the leaf is borne; at first the larger leaves have longer petioles, while those four to six internodes above the rosette begin to have shorter petioles the longer the internodes. No such influence of position, other than order in series, could be determined in regard to the leaf character employed here, either by inspection of the plants themselves or by the curves obtained.

The larkspur leaf is divided basically on a trichotomous pattern. All the first leaves, after the cotyledons, had at least three sections, and these three basic sections are retained in all subsequent, more incised leaves (fig. 1). Within each of the three basic sections, subsequent trichotomous division in the older leaves may be decidedly imperfect. In leaf 3, figure 1, it may be seen that, although the middle section (M) and one side (Sa) are di-

vided again just three times, in the other side (Sb) the lowermost of the three components is split again into two points. In the older leaves, patterns even more divergent from the simple trichotomous type are found.

Although the total number of points progresses smoothly from leaf to leaf (fig. 2*T*), it was apparent that this increase did not maintain the same proportion of points between the middle section and the two sides. When the average of the points per side is plotted alongside the average of the points per middle section against leaf number (fig. 2*S* and *M*, respectively), the change in ratio is clearly obvious. The shape of curves *S* and *T* of figure 2 seems to suggest a sigmoid sequence with upper inflections beginning at leaf 8.

Logarithmic plotting of the average number of points for the sides against the average for the middle section yields an even more irregular curve than that for either of these two variables plotted against leaf number (fig. 3). The shape appears to be doubly sigmoid, the lower section from leaf 1 to leaf 7, the upper from leaf 7 to leaf 10. A straight line drawn by inspection yields a slope or k value in the HUXLEY (2) equation for relative growth¹ of 1.23. Table 1 gives the nine progressive k values calculated from the logarithms of the averages for the slopes of the straight lines connecting leaf 1 with leaf 2, leaf 2 with leaf 3, etc. In view of the recent evidence showing that the consequences of sigmoid growth may (4) but not necessarily (3) lead to wide differences from the linear of relative growth curves plotted logarithmically, the k values calculated for the individual sections of the curve must not be considered as actual changes in the HUXLEY relative growth constant or in

¹ $y = bx^k$.

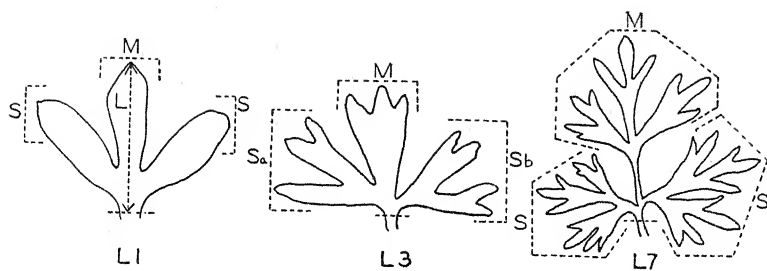
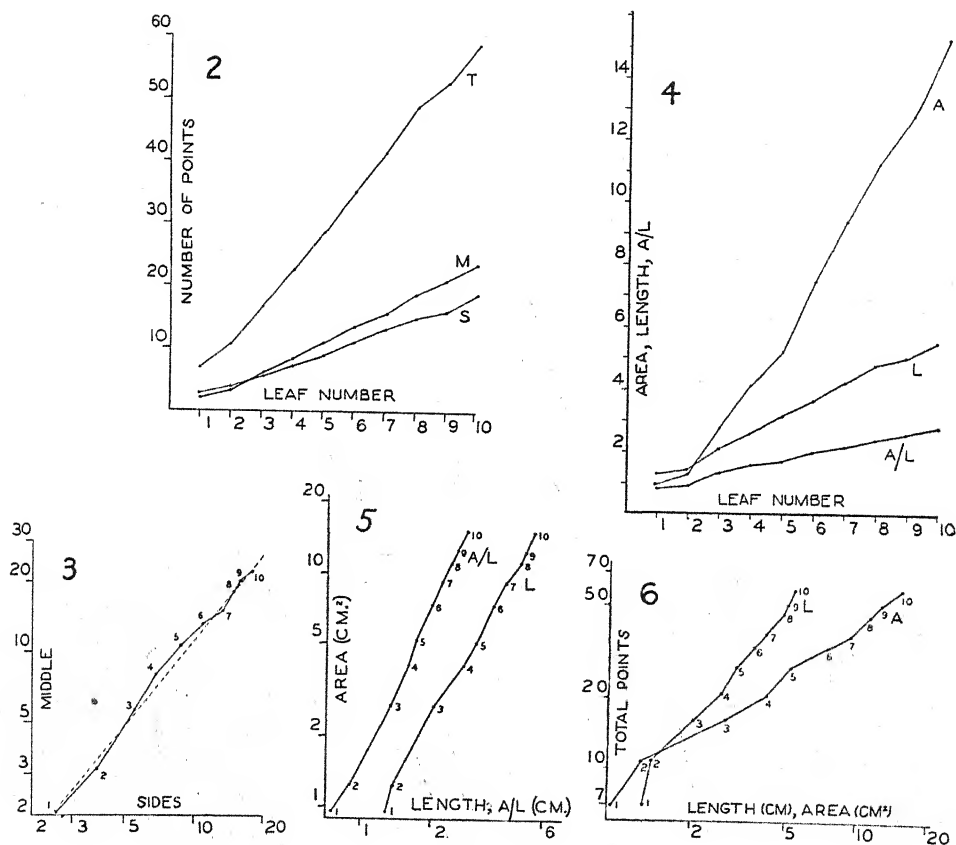


FIG. 1.—Three leaves from plant no. 32a16, redrawn from projection outlines: leaf 1, $\times 2\frac{1}{2}$; leaf 3, $\times 1\frac{1}{4}$; leaf 7, $\times \frac{1}{8}$. *S*, points included in counts per side (*Sa*, *Sb*, see text); *M*, points included in counts per middle; *L*, length measurement.



FIGS. 2-6.—Variation and relative development of several characters in leaves 1-10. All points connected by straight lines. Fig. 2, number of points: *T*, total; *M*, middle; *S*, side; against leaf number; $N=34-36$ for each leaf number. Fig. 3, points per middle against points per side; logarithmic; leaf numbers given along curve; $N=34-36$ for each leaf number. Dotted line is average slope from inspection (slope = 1.23). Fig. 4, area, length, and area/length against leaf number; $N=26-28$ for each leaf number. Fig. 5, area against length and area/length; logarithmic; leaf numbers given along curve; $N=26-28$ for each leaf number. Fig. 6, total points against length and area; logarithmic; leaf numbers given along curve; $N=26-28$ for each leaf number.

other growth constants not considered here.

The three other characters studied—area, length, and area/length—also suggest, but less definitely, a sigmoid type of development when plotted against leaf number (fig. 4). When area/length and length are plotted against area on a log-log grid (fig. 5), the relative growth curve takes on a more decided sigmoid aspect. The steep slopes, approximating 2, of both these curves are to be expected when growth in two di-

points must be regarded as expressing the number of segments into which the area is divided, rather than segments resulting from splits engendered by increase in circumference. If a certain rather large minimal increase in area is required before a new segmentation will occur, a broken relative growth curve would be expected, as was actually found. In this fashion, the shape of the curves in figure 6 may be accounted for without assuming repeated radical changes in growth constants for which there is no apparent physiological basis.

TABLE 1

INDIVIDUAL k^* VALUES BETWEEN
SUCCESSIVE LEAVES

Leaf numbers.....	k
1 and 2.....	1.08
2 and 3.....	1.47
3 and 4.....	1.58
4 and 5.....	1.27
5 and 6.....	1.01
6 and 7.....	0.78
7 and 8.....	1.48
8 and 9.....	2.32
9 and 10.....	0.60
Unweighted average.....	1.29

* In the HUXLEY (2) relative growth equation,
 $y = bx^k$.

mensions is plotted logarithmically against growth in one. The more emphatic sigmoid trends observable in the logarithmic plots (figs. 3, 5) indicate that, had it been possible to utilize absolute rather than physiological time intervals for arithmetic plotting, these curves themselves would have been more obviously sigmoid.

Logarithmic plotting of area and length against total number of points (fig. 6) yields the interesting observation that the number of points increases directly with the area and exponentially with the length, and thus exponentially with the circumference or any other linear dimension. Therefore, the number of

Discussion

The most interesting observation to be made on the material reported here is that the fully developed leaves of larkspur bear a relationship to one another in the characters analyzed typical of developmental sequences in single organs. There is no a priori reason that this form of development would be followed, and much additional work must be done before it can be assumed generally true. The best interpretation at hand is that the growing point itself changes in a regular fashion, and that this regular change is reflected in the mature leaves. Thus an analysis of the curve of changes in growing point size is indicated as a basis for further understanding of problems of this type.

The field to be analyzed may be conveniently divided into three major aspects: (1) developmental sequence in the growing point; (2) development of the homologous organs formed from the growing point; (3) maturation of the homologous organs.

Studies combining data relevant to all these aspects will be necessary to explain sufficiently the observations currently reported. Only the development of the homologous organs has heretofore been

analyzed to a satisfactory extent. ABBE *et al.* (1) have shown that development in homologous organs is parallel. WHALEY (6) states that organ size may be directly correlated with meristem size at any stage during development; however, no data were given which could be used to distinguish complete dependence of organ on the growing point from at least partial autonomy of the individual organ in length of time of development. That the leaf may have considerable autonomy in some respects is indicated by WHALEY'S (7) analysis of the change in shape during individual development of leaves of *Tropaeolum* arising from presumably mature meristems. Consequently only two factors, environmental changes and differences in the completion of maturation, prevent the mature leaves from being direct reflections of the changes in growing point size. The effects of environmental changes are obvious. For example, leaf 1 may be completely matured, while leaf 6 suffers from the effect of an environmental change which does not affect leaf 8 because it has not yet appeared.

SINNOTT (5) has shown that difference in fruit shape between two races of gourds, both continuing along the same course of heterogonic development, is due simply to the attainment of a larger size by one race than by the other. Such size differences, or differences in length of developmental period, would yield variation in the homologous organ series not traceable to the growing point. Although not likely in the case of number of points (the same in the young as in the mature leaves), and consequently of the other characters showing a direct relationship to point number, effects of this sort might possibly be operating in all the characters analyzed here. Finally, if longer developmental periods of the in-

dividual leaves were staggered symmetrically, the influence on the shape of the curve would not be obvious without biological criteria. The simplest way of solving this last problem is again by a study of growing point changes, comparing them with the mature leaf sequences.

A final point of interest is the bearing of studies on the developmental relationships of leaves to a solution of the stem-leaf versus the shoot-complex interpretation of plant structure. As yet biologists have devised no satisfactory criteria for measuring degree of differentiation. Yet in deciding whether a given heterogeneous structure should be regarded as composed of two distinct organs (here leaf and stem) or of a single organ of two parts (here shoot-complex), the solution rests ultimately on the interdependence of growth and differentiation in the two parts. If an organism is to be symmetrical, capable of functioning as an integrated whole, each part must normally bear a definite developmental relationship—within rather broad limits—to every other part. The technique of relative growth measurements seems to offer the best method for a comparative study of the magnitude of developmental interdependencies.

Summary

1. Mature leaves of larkspur, for the aspects studied, bear a relationship to one another typical of growth sequences in single organs.

2. Slightly sigmoid curves were obtained when the characters (number of points per side, per middle, total, and area, length, and area/length) were plotted against physiological time intervals (leaf number).

3. Log-log plotting of some characters against others yields relative growth re-

lationships expected from sigmoid developmental sequences.

4. The problem of the developmental factors underlying these relationships is briefly analyzed.

5. The number of points is in direct relationship to the area of the leaf, indicating that incision must be regarded as a segmentation of the area rather than a splitting in from the circumference.

6. The use of homologous organ series

in problems of relative growth and the use of relative growth techniques in estimating degree of differentiation are discussed.

The writer is indebted to the Horticulture Department for greenhouse facilities.

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APPLICATION OF GROWTH-REGULATING SUBSTANCE IN AEROSOL FORM, WITH SPECIAL REFERENCE TO FRUIT-SET IN TOMATO

CHARLES L. HAMNER,¹ HAROLD A. SCHOMER,² AND PAUL C. MARTH³

Introduction

Many methods have been employed in applying growth substances for obtaining parthenocarpic fruit-set on tomatoes, including spraying the flowers with lanolin emulsions containing the substances, smearing the styles with lanolin-growth substance paste, or applying vapors of growth substances to the flowers (3, 6, 8). HOWLETT (5) has

effectively used lanolin emulsions as carriers in increasing fruit-set, size, and quality. The most effective growth substance reported by him was indolebutyric acid. He has recommended that the treatment should follow pollination and be near the end of full bloom. This method does not result in the production of seedless fruit but increases fruit-set, size, and quality. An atomizer is employed to spray each individual flower; hence large-scale operations may be difficult. MITCHELL and WHITEHEAD (7) found that under greenhouse conditions β -

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naphthoxyacetic acid vaporized on a hot plate resulted in the development of 50-98% seedless fruits. Plants of the Marglobe variety were successively fumigated as the earliest flowers of each cluster opened.

Recently a new method of hormone application has been used (4), based on the aerosol principle devised by GOODHUE (2) for applying insecticides. In the initial experiments, β -naphthoxyacetic acid applied by this method was very effective in stimulating the production of seedless fruits and in increasing the number set. This paper reports further studies of the aerosol method, using β -naphthoxyacetic acid and several other growth substances, tested at many concentrations and under various conditions.

The method of aerosol production generally employed in these experiments is a simple one. The growth-regulating substance is first dissolved in some compound in which it is readily soluble and then added to a highly volatile liquefied gas. If the substance is soluble in the liquefied gas, it may be added directly to it. In either case, this mixture of liquefied gas, growth substance, and carrier solvent must be held under pressure in a container which is equipped to release the mixture as a very fine mist. After such release, the carrier promptly volatilizes and leaves the growth substance suspended in the atmosphere as an exceedingly finely divided liquid or solid.

In developing the method, it was necessary first to find a solvent for the naphthoxyacetic acid having a boiling point so high that, when sprayed, the acid would remain in solution in the finely dispersed droplets rather than fall out of solution as a solid or as dry crystals suspended in the air. Cyclohexanone (boiling point 155° C.) was finally determined upon as a suitable solvent. Others—such

as acetone and carbitol—were tried, but cyclohexanone was selected because it readily dissolved many growth-regulating substances and it had also proved very effective in insecticidal aerosols.

It was necessary also to secure a liquefied gas readily miscible with the cyclohexanone and solution of growth substance and yet one which after release would evaporate almost instantaneously. Methyl chloride⁴ (boiling point -10.57° F.) and dichloro-difluoromethane (Freon) (boiling point -21.7° F.) were first tested, but neither was miscible with this particular growth substance dissolved in cyclohexanone. Methyl ether (dimethyl ether) (boiling point -9.6° F.), however, proved a satisfactory solvent for cyclohexanone and the acid, and was miscible with the cyclohexanone.

The vapor pressure of suitable liquefied gases is sufficient to disperse the solution into fairly small droplets as it emerges from the spray nozzle. The droplets are probably still further subdivided by the violent boiling of the liquefied gas remaining in the droplets. By the time all the gas has completely disappeared from the droplets, most of them are so small that they settle out of the air very slowly.

The initial testing of the solubilities of various growth-regulating substances in these liquefied gases was done by L. D. GOODHUE of the Bureau of Entomology and Plant Quarantine. For this purpose two cylinders (fig. 1) were designed (2). The pure liquefied gas from a large stock container was transferred to the small steel cylinder (fig. 1A) by means of a short loading tube. The glass test-tube of the second cylinder (fig. 1B) was then removed by unscrewing the plug from the lower end. Into this tube the desired amount of growth substance or its solu-

⁴ E. I. du Pont de Nemours and Company.

tion was put. The tube was then replaced in the cylinder and tightened into position by means of the screw at the base. The spray nozzle was replaced by a hexagonal nipple, and—with the valve open—the air in the tube was evacuated by means of a suction pump. Following evacuation, the valve was closed and

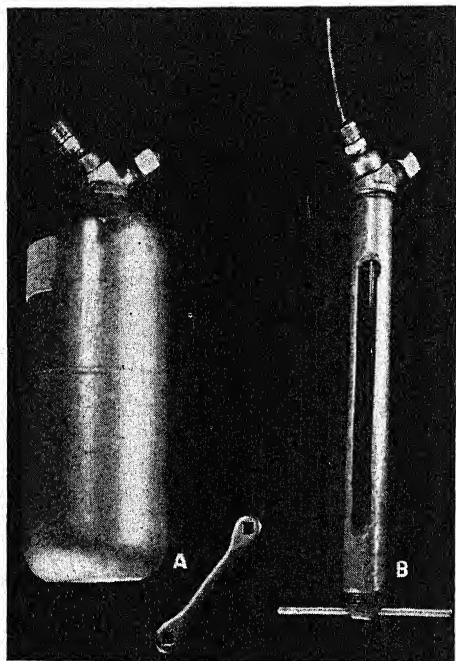


FIG. 1.—Apparatus for aerosol treatments: A, steel cylinder with spray nozzle and wrench; B, testing tube with fine capillary nozzle.

the two cylinders connected by means of the nipple. The valves were opened on each cylinder, thus allowing the liquefied gas to flow from A to B. If sufficient solvent failed to move, hot water was applied to A, thereby increasing the vapor pressure in that cylinder. Ten grams of solvent was forced into the glass test-tube and the solubility determined. If the substances were insoluble, a cloudy precipitate formed. β -naphthoxyacetic acid, indolebutyric acid, indoleacetic

acid, and α -naphthaleneacetic acid are all soluble, at least up to 1% in solutions consisting of 10% cyclohexanone and 89% methyl ether.

In applying the methyl ether-cyclohexanone solution of growth substance as an aerosol to tomato flowers in amounts up to 500 gm., a cylinder similar to that of figure 1A may be used. If only a few plants are treated, the cylinder with glass test-tube (fig. 1B) is suitable. When using either cylinder, the valve is opened with a wrench (fig. 1), a spray nozzle may be attached (fig. 1B), and the amount of solution used in a given time determined by accurately weighing the cylinder before opening and after closing the valve. The nozzle delivery rate is calibrated by this method.

Investigation

EXPERIMENT I: FUMIGATION METHOD OF APPLYING β -NAPHTHOXYACETIC ACID AS AN AEROSOL

The term "fumigation" as used in this paper refers to the use of the aerosol as a vapor treatment, in which the aerosol is released into a closed room in which everything is exposed to the treatment.

For the tests of the effect of fumigation with an aerosol of naphthoxyacetic acid dissolved in cyclohexanone and methyl ether, Pan America tomato plants were grown in 8-inch pots under greenhouse conditions, with temperatures maintained at 65°–70° F. at night and 70°–75° F. during the day. When they were approximately 18 inches tall (fig. 2) and the first flower of the first cluster had opened, ninety-six of the most nearly uniform plants were chosen and forty-five of these placed in a closed room some distance from the greenhouse. They were then treated with aerosol by releasing a mixture supplying 240 mg. naphthoxy-

acetic acid, 21.36 gm. methyl ether, and 2.4 gm. cyclohexanone per 1000 cu. ft. The plants were treated at 4:00 P.M. October 16, 1943, and were taken out of the room at 8:00 A.M. the following day. They were then placed on the greenhouse benches, together with the control plants which had not been treated.

RESULTS.—Five days after treatment the plants that had received the aerosol



FIG. 2.—Maturity of tomato plant at time of first treatment with aerosols.

treatment had 123 fruits (table 1), or an average set of about three fruits per plant, and at this time the largest of them were about $\frac{1}{2}$ inch in diameter (fig. 4). Although the clusters were treated when only the first flower had opened, many of the unopened flowers set fruits, and these enlarged rapidly. The fifty control plants (fig. 3) at this time had set but two fruits. The style persisted much longer on the treated than on the control fruits and showed some enlargement. Likewise some of the petals persisted 3-4 days longer on the treated than on the untreated flowers.

As the fruits ripened they were removed from the plant, weighed, and sliced to determine the relative seed development and general condition. The treated tomatoes ripened earlier and were larger in diameter and heavier than the controls (table 2), and most of them were seedless. In a few, however, the ovules had undergone partial development, the amount varying with the condition of the flower when treated. The flowers that were open at the time of treatment showed greater ovule develop-

TABLE 1

NUMBER OF FRUITS SET ON FIRST CLUSTER OF TOMATO PLANTS, OCTOBER 21, 1943, 5 AND 9 DAYS AFTER TREATMENT BY FUMIGATION WITH β -NAPHTHOXYACETIC ACID AS AEROSOL

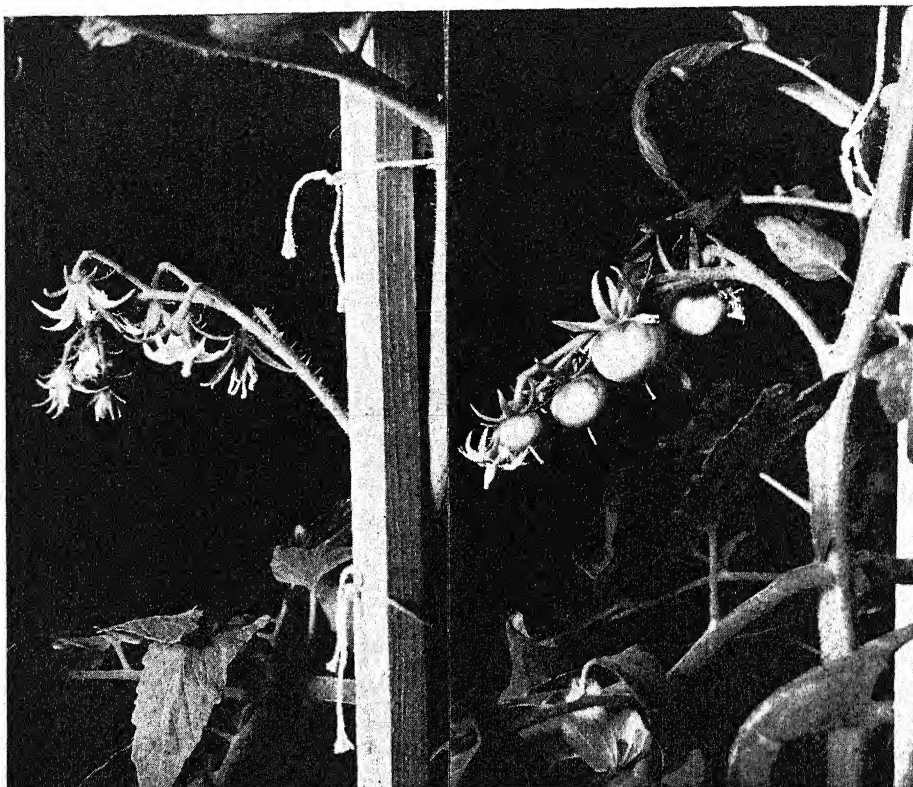
OBSERVATION DATE	TREATED		CONTROLS	
	No. of plants	No. of fruits set	No. of plants	No. of fruits set
October 21..	45	123	50	2
October 25..	45	123	50	22

ment than did those treated prior to opening of the flowers.

EXPERIMENT II: DIRECT APPLICATION OF AEROSOL

Spraying of the aerosol differs from the fumigation treatment in that the aerosol mixture is sprayed directly at the open flower cluster (fig. 5), necessitating no inclosed chamber or removal of the plants from their original positions. In this respect the operation resembles any ordinary water-spray method.

Three varieties of tomatoes—Pan America, Marglobe, and Bonny Best—were grown under greenhouse conditions during February, 1944. At the time the first cluster of flowers had opened, sixty plants were chosen from each of the varieties and these divided into five lots



FIGS. 3, 4.—Fig. 3 (left), untreated plant 5 days after treatment was administered to plant shown in fig. 4. Fig. 4 (right), 5 days after treatment of cluster with 1% β -naphthoxyacetic acid used as aerosol in fumigation method.

TABLE 2

DATES OF RIPENING AND WEIGHTS OF MATURE FRUITS FROM PLANTS TREATED BY FUMIGATION WITH β -NAPHTHOXYACETIC ACID AS AEROSOL, AND FROM UNTREATED CONTROLS. TREATMENT OCTOBER 16, 1943, WHEN FIRST FLOWER OF FIRST CLUSTER WAS OPEN

HARVESTED (1943)	AEROSOL-TREATED			CONTROLS		
	Av. wt. of fruits (gm.)	Av. diameter of fruits (inches)	No. of fruits	Av. wt. of fruits (gm.)	Av. diameter of fruits (inches)	No. of fruits
11/27.....	175.7	3.07	12	130	2.71	1
11/29.....	187.3	3.00	16	68	2.01	1
11/30.....	207.0	3.13	5	96	2.83	1
12/1.....	193.0	3.08	9	165	2.82	3
12/2.....	211.0	3.14	14	96	2.23	4
12/3.....	167.0	2.83	15	116	2.45	4
12/4.....	158.0	2.75	8	111	2.39	5
12/6.....	151.0	2.68	12	148	2.60	7
12/7.....	161.0	2.72	5	145	2.59	3
12/9.....	158.0	2.77	4	120	2.45	11
12/11.....	154.0	2.79	3	114	2.42	4
12/13.....	130.0	2.51	3	110	2.39	13

of twelve plants each. The first three lots received 1% concentrations of 4-chlorophenoxyacetic, naphthoxyacetic, and in-

without growth substance. The fifth was a control which received no spray treatment.

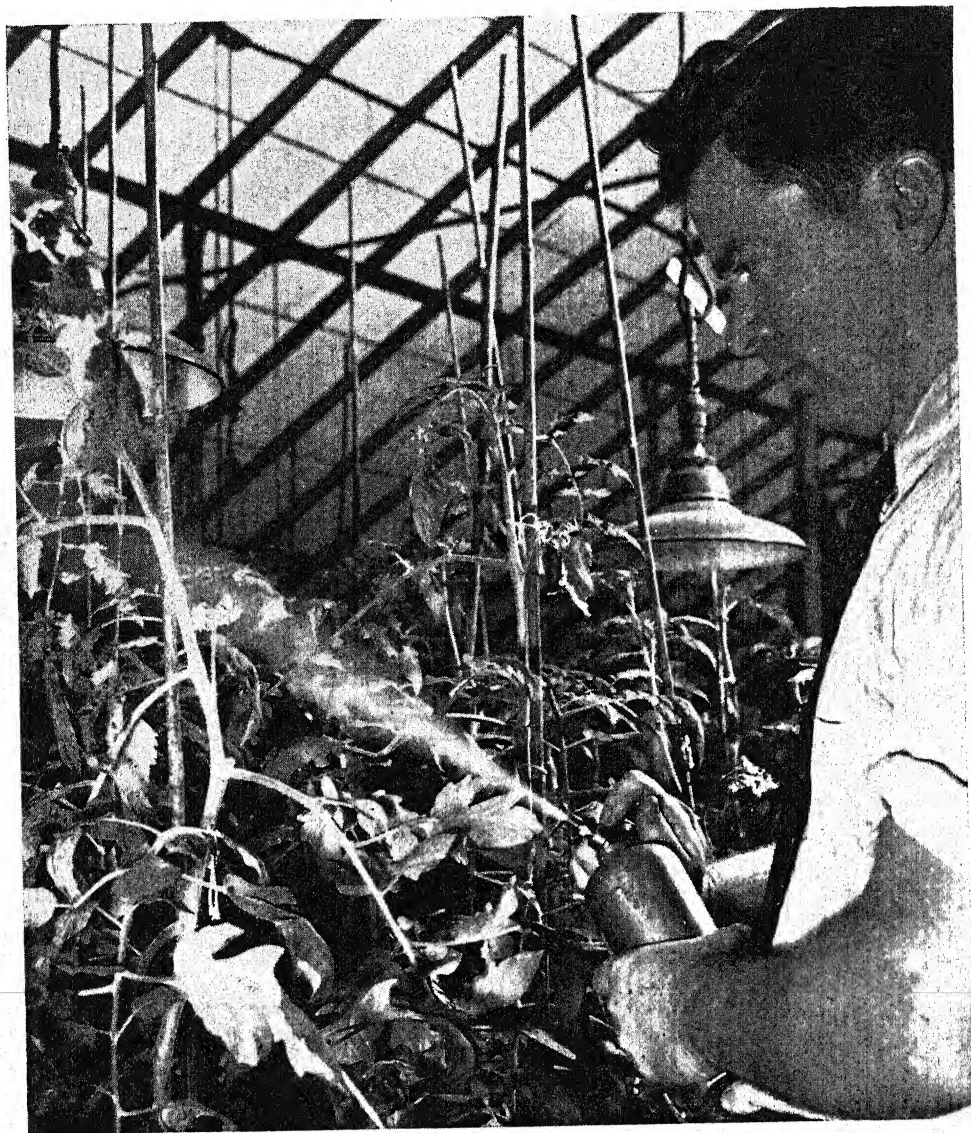


FIG. 5.—Direct application of aerosol. Steel cylinder held approximately 1 foot from flower cluster, valve then opened for a second, and a fine mist envelops cluster. This is sufficient to insure a high percentage of set.

dolebutyric acid, respectively, in the form of cyclohexanone-methyl ether aerosols. The fourth lot received aerosol

Each lot of plants was successively taken out of the greenhouse into a well-ventilated room. The number of flowers

and buds on each plant was recorded and the plants lined up in a row. The treatment was applied directly to the individual clusters by holding the cylinder about 1 foot away, releasing the valve, and rapidly spraying each cluster with the vapor. The thirty-six plants in each treatment received a total of 12

so arranged that two plants per treatment of each variety were placed on each bench, making a total of thirty plants per bench. The plants were then allowed to grow under greenhouse conditions. Records were taken at weekly intervals of the size of the fruits and the number of fruits set. The fruits were harvested at

TABLE 3

EFFECT OF GROWTH SUBSTANCES APPLIED AS AEROSOL SPRAY ON FRUIT-SET. CONCENTRATION OF COMPOUND IN AEROSOL WAS 1%; OF CYCLOHEXANONE, 10%; AND OF METHYL ETHER, 80%. TREATMENT FEBRUARY 5, 1944

Treatment	Total no. flowers and buds treated	Total no. flowers open when treated	Total no. fruits set on 2/14/44	Av. diameter of fruits on 2/14/44 (inches)	Total no. fruits set on 2/28/44	Av. diameter of fruits on 2/28/44 (inches)	Av. wt. at harvest (gm.)
Bonny Best							
4-Chlorophenoxyacetic acid	57	27	35	0.7	40	1.6	99.3
Naphthoxyacetic acid.....	62	27	16	0.7	20	1.9	96.8
Indolebutyric acid.....	61	26	7	0.3	12	1.4	78.9
Aerosol control.....	60	27	5	0.4	14	1.5	88.0
Untreated.....	56	28	14	0.6	15	1.3	78.0
Marglobe							
4-Chlorophenoxyacetic acid	57	26	32	0.7	37	2.0	141.8
Naphthoxyacetic acid.....	59	27	22	0.7	24	1.8	135.0
Indolebutyric acid.....	53	24	15	0.5	20	1.8	120.3
Aerosol control.....	62	27	12	0.4	20	1.7	110.9
Untreated.....	60	24	18	0.5	17	1.5	139.0
Pan America							
4-Chlorophenoxyacetic acid	53	31	32	0.6	44	2.0	128.6
Naphthoxyacetic acid.....	58	28	16	0.8	18	2.3	177.0
Indolebutyric acid.....	67	30	7	0.3	17	1.2	80.5
Aerosol control.....	53	29	5	0.3	13	1.2	92.3
Untreated.....	58	23	5	0.3	13	1.4	108.8

gm. of aerosol, or 0.33 gm. of solution containing 3.3 mg. of growth substance per plant. In all probability only a small fraction of this amount was deposited on the clusters, of course. After each respective treatment, the doors and windows of the room remained open to disperse the aerosol before bringing in the plants for the application of any succeeding treatment.

After treatment, the plants were returned to the greenhouse and placed at random on six greenhouse benches, being

the late-pink to full-red stage of maturity, at which time the final size, weight, and condition were recorded. The control plants received the same amount of handling, except that no aerosol treatments were given. It was thought that the control plants were jarred enough to permit pollination to take place without hand pollination.

RESULTS.—In all three varieties, the flowers that received 4-chlorophenoxyacetic acid at 1% concentration set more fruit than did the flowers receiving

other treatments (table 3). There were in fact more fruits set per plant than there were open flowers at the time of treatment, since many of the unopened buds developed fruit directly. With the chlorophenoxyacetic-acid treatment, a total of ninety-nine fruits had set 9 days after treatment, an average of 2.4 per cluster. Twenty-three days after treatment 121 fruits had set, an average of 3.3 per cluster. On the control plants a total of 37 fruits had set 9 days after treatment, an average of 1.0 per cluster. The fruits from the control plants had an average diameter of 0.44 inch as compared with 0.67 inch for the chlorophenoxyacetic acid-treated fruits. A 1% solution of this acid was injurious, and the leaves developed various deformities. The treated fruits at the time of harvest were in some cases misshapen (fig. 6), many had developed rot, and the placentas of some had failed to develop and fill the carpellary cavities. No seeds were present in any of the treated fruits.

Flowers treated with 1% naphthoxyacetic acid developed many seedless fruits. In contrast with those resulting from treatment with 4-chlorophenoxyacetic acid, these fruits were excellent in texture and the locules were well filled. Although most of the fruits were entirely seedless, a few had occasional seeds. Early growth of the fruits was greatly stimulated by treatment with this compound. Later experiments showed, however, that a greater set can be obtained when the concentrations are lower than 1%.

Flowers treated with an aerosol of indolebutyric acid at 1% concentration developed seeded fruits, and no increase in fruit-set or in the total number of fruits per cluster was noted. In another experiment with the Marglobe variety,

aqueous lanolin-emulsion sprays containing 0.2% indolebutyric acid resulted in a significantly higher fruit-set over control plants.

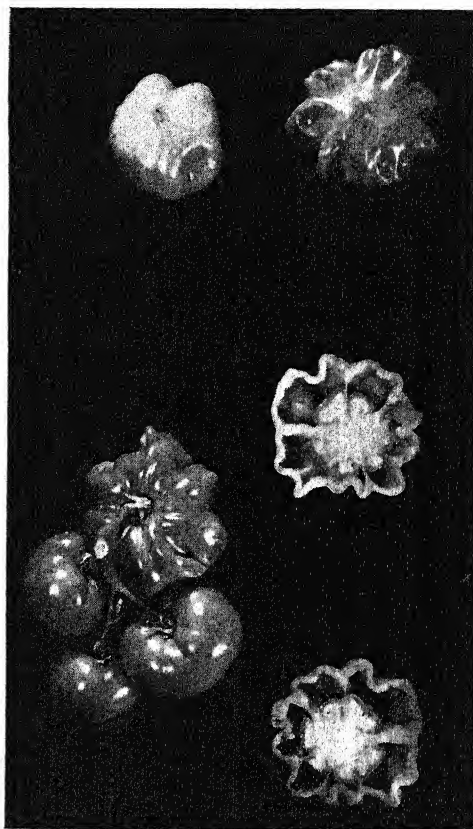


FIG. 6.—Deformed tomatoes resulting from treatment with 4-chlorophenoxyacetic acid at 1% concentration. Carpel walls have developed, but placentas have developed slowly so that locules are not well filled.

EXPERIMENT III: AEROSOL APPLICATIONS TO SUCCESSIVE CLUSTERS OF FLOWERS

Plants of Rutgers variety were started at the same time as the three varieties used in the preceding experiment, but these plants grew more slowly and as a result the first flowers opened about a week later. Since no data had then been

obtained from the previous experiment, the same growth substances and concentrations were used. Each successive

Twelve plants were used for each of the five treatments. Application to the first clusters was made February 7, and

TABLE 4
EFFECT OF AEROSOL SPRAY AT CONCENTRATIONS OF 1% ON FRUIT-SET OF
SUCCESSIVE CLUSTERS OF RUTGERS TOMATO. SUCCESSIVE APPLICATIONS ON FEBRUARY 7, FEBRUARY 24, AND MARCH 15, 1944

Treatment	Cluster number	No. of flowers and buds treated*	No. of flowers open*	No. of fruits set	Av. diameter of fruit-set at time of harvest (inches)
4-Chlorophenoxyacetic acid	1.....	59	30	40	2.46
	2.....	76	61	40	2.18
	3.....	47	26	25	2.10
	4.....	40	29	10	1.91
	5.....	20	12	6	1.17
Total.....		242	158	121	2.18†
Naphthoxyacetic acid	1.....	60	26	14	2.72
	2.....	69	23	42	2.25
	3.....	65	39	17	1.81
	4.....	38	35	6	1.56
	5.....	19	16	5	1.20
Total.....		251	139	84	2.13†
Indolebutyric acid	1.....	63	31	17	2.86
	2.....	80	72	15	2.24
	3.....	67	33	12	2.21
	4.....	60	51	9	2.14
	5.....	26	16	3	0.83
Total.....		296	203	56	2.33†
Aerosol control, cyclohexanone+dimethyl ether	1.....	61	27	10	2.37
	2.....	55	34	9	2.35
	3.....	62	42	5	2.30
	4.....	46	46	10	1.43
	5.....	23	20	7	1.47
Total.....		247	169	41	1.97†
Untreated	1.....	58	28	10	2.67
	2.....	67	60	14	2.02
	3.....	61	36	18	2.20
	4.....	54	44	9	2.00
	5.....	34	26	4	1.18
Total.....		274	194	55	2.13†

* Totals for twelve plants.

† Average.

cluster was treated when many of the flowers were open to study the effect of repeated applications of aerosols of growth-regulating substances on individual plants.

the plants were then equally divided on two greenhouse benches. The next application, for the second and third clusters, was made February 24. On March 13 the fourth and fifth clusters were treated.

For each treatment the plants were removed from the greenhouse to a small ventilated room and sprayed as in experiment II. Not all the plants had clusters

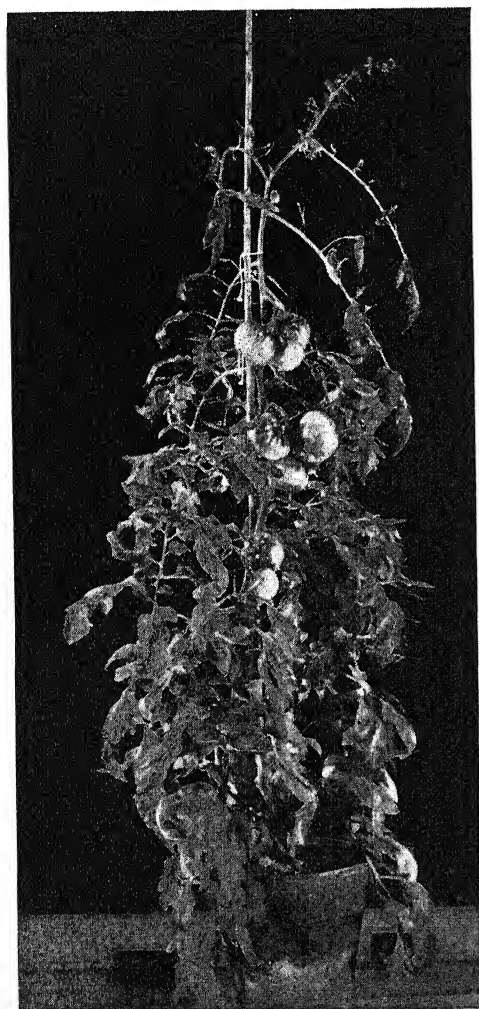


FIG. 7.—Three successive clusters of fruits derived from flowers treated February 7 and February 24 with aerosol of 4-chlorophenoxyacetic acid at 1% concentration.

in the same stage of development. Some had flowers which were classified as over-mature; that is, the petals were dry and the style had fallen off. Other flowers either had not yet opened or were in the bud stage.

RESULTS.—With the Rutgers variety (table 4), 4-chlorophenoxyacetic acid, at 1% concentration, was again the most effective compound in stimulating fruit-set, but here also many cavities were found in the mature fruits and increased decay was noted. Naphthoxyacetic acid, as in the previous experiment, was also very effective. Flowers sprayed with indolebutyric acid showed very little response; no increase in set of fruits was noted, and of the fruits that set only a very few were seedless.

The Rutgers plants treated three times with 4-chlorophenoxyacetic acid (fig. 7) set an average of 3.3 fruits per cluster on the first two clusters and an average of 2.1 on the third cluster. The fourth and fifth clusters had an average of less than one fruit per cluster. This may have resulted in part from the fact that the 1% concentration of the acid was so high that much bud inhibition and malformation of the leaves resulted. The fruits of all clusters, although usually normal in appearance, were not well filled, and in some cases decay in the center had developed. Occasionally ovaries of flowers that were classified as over-mature were stimulated and partially seedless fruits developed. Seeds often developed in only one locule. The two control lots averaged less than one fruit per cluster.

EXPERIMENT IV: CONCENTRATION OF GROWTH REGULATORS IN AEROSOL SOLUTIONS

It was apparent from results obtained in experiments II and III that the concentration of growth substance in the aerosol solution was too high, and four varieties of tomatoes—Bonny Best, Marglobe, Rutgers, and Pan America—were used to test the effect of concentration. Five growth substances—4-chloro-

phenoxyacetic acid, β -naphthoxyacetic acid, indolebutyric acid, indoleacetic acid, and α -naphthaleneacetic acid—were applied, each at concentrations of 0.05, 0.1, and 0.5%. The plants received these treatments when two or three flowers of the first cluster had opened. Ten plants from each variety were used for each treatment. The aerosol solution was prepared in a pressure test-tube similar to that of figure 1B, but of larger capacity. The weighed amounts of growth substances were dissolved in 0.75 gm. of cyclohexanone. This solution was placed in the test-tube and 14.25 gm. of methyl ether forced into the tube as previously described. Approximately 0.5 gm. of the aerosol was used for each cluster. The spraying operation was similar to that shown in figure 5. Before the plants were treated, the total numbers of flowers and buds per plant were recorded, as well as the number of open flowers. Ten days after treatment the fruits were harvested. At this time the number and size as well as the internal condition of the fruits (seeds or seedless) were recorded.

RESULTS.—All three concentrations of 4-chlorophenoxyacetic acid were effective in stimulating the set of fruit (table 5). At concentrations of 0.5 and 0.1%, injury and deformity of the stem and leaves were noted; the 0.05 concentration resulted in only very slight deformation of the leaves. All four varieties responded to this 0.05% treatment, and early growth of the fruits was significantly stimulated. Ten days after treatment, all the young fruits were seedless. The control fruits at this stage had visible (determinable) seeds.

Naphthoxyacetic acid was also very effective, and no injury or formative effects were noted at any of the concentrations tested. When applied at 0.05%

it was effective in setting fruit on Pan America, Marglobe, and Rutgers, but it was not very effective in setting seedless fruit on Bonny Best. This concentration seemed to be near the lower limit of effectiveness.

Indolebutyric acid, indoleacetic acid, and α -naphthaleneacetic acid were not consistently effective in increasing fruit-set or in inducing development of seedlessness with any of the four varieties. Naphthaleneacetic acid when applied at 0.5% concentration inhibited fruit-set and enlargement.

EXPERIMENT V: OTHER DILUTIONS OF 4-CHLOROPHENOXYACETIC ACID

The previous experiments using 4-chlorophenoxyacetic acid at 0.5, 0.1, and 0.05% had caused injury and inhibition; hence greater dilutions were necessary. Three concentrations were used—0.01, 0.001, and 0.0001% in a methyl ether-cyclohexanone aerosol. Three varieties, Bonny Best, Pan America, and Rutgers, were employed. The plants were grown during March, with ten plants of each variety in each treatment. The first clusters were treated by direct spray shortly after the flowers began to open. Ten comparable plants of each variety were used as controls.

RESULTS.—No injury was noted to leaves or stems at any of the three concentrations. A significant increase in set over the controls was found with all concentrations (table 6). A greater set, however, was recorded at 0.01% than at either 0.001 or 0.0001%. Some of the fruits that developed in the treatment with 0.0001% contained seeds, indicating that the lower limit of effectiveness was reached. It was also noted that even at the very low dilutions some of the fruits were poorly filled.

TABLE 5

EFFECT OF CONCENTRATIONS OF GROWTH-REGULATING SUBSTANCES AS AEROSOLS ON
FRUIT-SET OF FIRST CLUSTER OF TOMATOES. GREEN FRUITS
HARVESTED 10 DAYS AFTER TREATMENT

Treatment	Total no. flowers and buds treated*	Flowers open when treated (%)	No. of fruits set*	No. of fruits with seeds*	No. of fruits without seeds*	Av. diam- eter of fruits 10 days after treatment (inches)
Bonny Best						
4-Chlorophenoxyacetic acid						
0.5%.....	48	44	29	0	29
0.1%.....	53	45	28	0	28
0.05%.....	53	40	12	0	12	0.8
Naphthoxyacetic acid						
0.5%.....	52	46	25	1	24	0.9
0.1%.....	45	56	28	3	25	0.8
0.05%.....	53	54	12	12	0	0.7
Indoleacetic acid						
0.5%.....	53	45	14	13	1	0.7
0.1%.....	52	42	16	14	2	0.7
0.05%.....	47	55	17	15	2	0.6
Naphthaleneacetic acid						
0.5%.....	45	38	6	0	6	0.7
0.1%.....	47	43	15	8	7	0.7
0.05%.....	57	44	17	16	1	0.7
Indolebutyric acid						
0.5%.....	52	42	14	11	3	0.8
0.1%.....	50	46	19	19	0	0.6
0.05%.....	50	44	16	14	2	0.8
Untreated.....	52	44	10	10	0	0.6
Marglobe						
4-Chlorophenoxyacetic acid						
0.5%.....	49	46.9	19	0	19	0.4
0.1%.....	49	48.9	22	0	22	0.8
0.05%.....	53	41.5	23	0	23	0.9
Naphthoxyacetic acid						
0.5%.....	52	42.3	18	3	15	0.9
0.1%.....	48	45.8	17	3	14	0.8
0.05%.....	48	52.0	21	0	21	0.8
Indoleacetic acid						
0.5%.....	46	41.3	5	5	0	0.6
0.1%.....	49	48.9	7	7	0	0.6
0.05%.....	39	55	8	8	0	0.5
Naphthaleneacetic acid						
0.5%.....	43	65	2	2	0	0.6
0.1%.....	46	59	0	0	0	0.5
0.05%.....	42	60	2	2	0
Indolebutyric acid						
0.5%.....	45	56	6	6	0	0.7
0.1%.....	41	60	8	8	0	0.8
0.05%.....	48	66	16	16	0	0.7
Untreated.....	52	42	8	8	0	0.6

* Totals for ten plants.

TABLE 5—Continued

Treatment	Total no. flowers and buds treated*	Flowers open when treated (%)	No. of fruits set*	No. of fruits with seeds*	No. of fruits without seeds*	Av. diam- eter of fruits 10 days after treatment (inches)
Rutgers						
4-Chlorophenoxyacetic acid						
0.5%.....	60	33	22	0	22	0.6
0.1%.....	57	39	26	0	26	0.6
0.05%.....	54	46	22	0	22	0.6
Naphthoxyacetic acid						
0.5%.....	58	38	17	1	16	0.9
0.1%.....	55	45	21	0	21	0.9
0.05%.....	56	43	22	0	22	0.8
Indoleacetic acid						
0.5%.....	38	55	7	7	0	0.8
0.1%.....	51	35	6	6	0	0.8
0.05%.....	49	41	6	6	0	0.8
Naphthaleneacetic acid						
0.5%.....	39	46	0	0	0	0.0
0.1%.....	31	48	5	5	0	0.6
0.05%.....	37	56	7	7	0	0.5
Indolebutyric acid						
0.5%.....	60	32	8	6	2	0.7
0.1%.....	62	21	10	10	0	0.5
0.05%.....	55	31	5	5	0	0.7
Untreated.....	40	43	5	5	0	0.6
Pan America						
4-Chlorophenoxyacetic acid						
0.5%.....	39	59	18	0	18
0.1%.....	42	55	23	0	23
0.05%.....	42	60	21	0	21
Naphthoxyacetic acid						
0.5%.....	50	38	17	0	17	0.7
0.1%.....	49	43	18	0	18	0.7
0.05%.....	54	28	15	0	15	0.8
Indoleacetic acid						
0.5%.....	51	55	4	4	0	0.5
0.1%.....	53	43	5	5	0	0.6
0.05%.....	45	56	4	4	0	0.6
Naphthaleneacetic acid						
0.5%.....	54	52	0	0	0
0.1%.....	50	58	0	0	0	0.5
0.05%.....	53	47	9	9	0	0.7
Indolebutyric acid						
0.5%.....	53	32	2	2	0	0.7
0.1%.....	50	28	6	6	0	0.6
0.05%.....	52	35	9	8	1	0.6
Untreated.....	50	42	5	5	0	0.5

EXPERIMENT VI: AEROSOL FUMIGATION WITH 4-CHLOROPHENOXYACETIC ACID

This experiment was designed to test the effect of an aerosol-vapor treatment, using two dosages of chlorophenoxyacetic acid. Three varieties were used—Bonny Best, Marglobe, and Pan America. The plants were grown during March and April until the first clusters each had

and allowed to grow. The young fruits were harvested 10 days later, and records of size and condition of seedlessness were recorded.

RESULTS.—Seedlessness and greatly increased fruit-set were obtained by using 4-chlorophenoxyacetic acid as a aerosol-vapor treatment (table 7). No injury to the foliage was noted at either

TABLE 6

EFFECT OF VARIOUS CONCENTRATIONS OF 4-CHLOROPHENOXYACETIC ACID AS AEROSOL ON SET AND DEVELOPMENT OF FRUITS OF TOMATOES. FRUIT HARVESTED 10 DAYS AFTER TREATMENT

Treatment	Total no. flowers and buds treated*	Total no. flowers open when treated*	Total fruit-set at end of 10 days*	Av. diameter of fruits 10 days after treatment (inches)
Bonny Best				
0.01%.....	46	13	23	0.5
0.001%.....	43	11	12	0.4
0.0001%.....	51	19	21	0.7
Untreated.....	52	17	6	0.4
Pan America				
0.01%.....	47	27	13	0.5
0.001%.....	50	28	15	0.7
0.0001%.....	55	32	11	0.5
Untreated.....	46	28	4	0.6
Rutgers				
0.01%.....	63	29	29
0.001%.....	62	31	22	0.9
0.0001%.....	59	26	23	0.9
Untreated.....	56	30	17	0.8

* Totals for ten plants.

two or three flowers open. Ten plants from each variety were used for each experiment. Two small rooms, capacity 1000 cu. ft., were employed, and into each room a group of thirty plants was taken. In one room an aerosol was released containing 25 mg. of chlorophenoxyacetic acid, in the other room one containing 50 mg. The same amount of methyl ether and cyclohexanone was released in each room. The plants were kept in the rooms for 16 hours and were then returned to the greenhouse benches

concentration. All three varieties responded to the treatment. Some of the plants exposed to the higher concentration of 50 mg. per 1000 cu. ft. showed slight epinasty. At greater concentrations the normal vegetative habit of the plant would probably be impaired. For the three varieties only thirteen fruits were set on a total of thirty untreated plants, while with the treatment at 50 mg. of chlorophenoxyacetic acid per 1000 cu. ft. of space, sixty-five fruits were set.

Discussion

Increased set of tomato fruits and in many cases production of seedless fruits can be obtained by application to the flowers of certain growth-regulating substances dispersed as aerosols. These compounds vary in their effectiveness. 4-Chlorophenoxyacetic acid was extremely effective, a concentration as low as 0.001% being sufficient to set fruit.

containers. A plant or tree can be treated in a few seconds merely by opening a valve. Very small amounts can be used for effective treatment and a uniform application made without injury or distortion of the plant if the correct concentrations are used.

Cyclohexanone itself applied in any large amount is toxic to tomato plants, but the small amounts deposited on the

TABLE 7

EFFECT OF 4-CHLOROPHENOXYACETIC ACID APPLIED AS FUMIGATION
ON SET AND DEVELOPMENT OF FRUITS OF TOMATOES.
FRUIT HARVEST 10 DAYS AFTER TREATMENT

Treatment	Total no. flowers and buds treated*	Total no. flowers open when treated*	Total no. fruits set after 10 days*	Av. diameter of fruits 10 days after treatment (inches)
Bonny Best				
25 mg./1000 cu. ft.....	53	23	19	0.7
50 mg./1000 cu. ft.....	47	26	26	0.7
Control.....	54	15	7	0.6
Pan America				
25 mg./1000 cu. ft.....	54	24	22	0.6
50 mg./1000 cu. ft.....	47	28	26	0.6
Control.....	53	26	4	0.5
Marglobe				
25 mg./1000 cu. ft.....	39	26	12	0.7
50 mg./1000 cu. ft.....	45	26	13	0.6
Control.....	59	30	2	0.6

* Totals for ten plants.

The locules of the fruits resulting from treatment with this substance, however, are usually poorly filled. Naphthoxyacetic acid is also very effective, but the necessary concentration appears higher than that of chlorophenoxyacetic acid. Indolebutyric acid and naphthaleneacetic acid have not been consistently effective, either in promoting fruit-set or in stimulating development.

The advantage of the aerosol method appears to arise from its rapidity and simplicity of application, once the materials have been prepared in suitable

plant by the aerosol treatment have shown no harmful effects.

HOWLETT (5) has reported increased set of fruit by the use of indolebutyric acid in an aqueous lanolin emulsion. We have repeated some of his experiments and have also noted an increased set of fruit. In an aerosol, however, indolebutyric acid appeared very ineffective in increasing fruit-set. The reason for this is not apparent.

Although these experiments have dealt largely with methyl ether as the vapor carrier, it is possible to use Freon,

either in a mixture of Freon and methyl ether together with the common growth substances, or of Freon with some of the esters of the growth substances. Methyl ether has been used largely because most of the growth substances are readily soluble in it. It has certain disadvantages, but for this purpose they are not great. At very high concentrations (50% or higher) in the atmosphere it is toxic to human beings. At 3% in the atmosphere it is inflammable. All of our work involved concentrations much lower than 3%.

The seedless tomato fruits developed by the use of aerosol method were of excellent quality. In experiments recently completed but not reported here, in which both 4-chlorophenoxyacetic acid at 0.01% and β -naphthoxyacetic acid at 0.25% were used together in aerosol, a very good set was obtained, and all the fruits were of excellent quality and well filled.

Summary

1. A recently developed aerosol method of applying growth-regulating substances to tomatoes has proved an effective means of inducing fruit setting and,

in many cases, of producing seedless fruits.

2. 4-Chlorophenoxyacetic, β -naphthoxyacetic, indoleacetic, indolebutyric, and α -naphthaleneacetic acids differed in their capacities to induce fruit-set. Of these, 4-chlorophenoxyacetic acid was the most effective in setting fruit and stimulating early fruit growth. When used at concentrations higher than 0.01%, the treated plants developed abnormal leaves and fruits.

3. β -naphthoxyacetic acid also induced setting of seedless tomatoes when applied as an aerosol. The safest and still effective range of concentration lies between 0.01 and 0.5%. Not all the fruits from treated flowers were seedless. Apparently their age and the amount of pollination determined to some extent the amount of seed development, but early fruit growth was greatly stimulated.

4. Indoleacetic, indolebutyric, and α -naphthaleneacetic acids were less effective than the other compounds in inducing fruit-set and producing seedless fruit when applied by this aerosol method.

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EFFECTS OF FLUORESCEIN AND PHOTOSENSIN ON GROWTH OF RED KIDNEY BEANS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 563

ROBERT K. ZUCK

Introduction

Many organic compounds are known to have local growth-regulating effects on plants. Attempts to disclose general stimulation of growth by these compounds have been undertaken many times, but critical work has revealed little or no stimulation of the growth of the entire plant by any of the compounds examined (2, 8). Among those tested were indoleacetic acid, indolebutyric acid, naphthaleneacetic acid, naphthalene acetamide, and phenylacetic acid. At the present time there is apparently no experimental evidence indicating stimulation of growth of the entire plant by this class of growth-regulating substances.

Recently it has been stated (6, 7) that fluorescein acts as an inhibitor and as a stimulator of plant growth, depending upon its concentration. This compound is not known to have local growth-regulating effects. In their work with fluorescein, SELLEI, SELLEI, MAYER, and WENT (7) have employed a commercial preparation (Photosensin) composed of 93.9% sodium bicarbonate, 5% fluorescein, 1% iron sulphate, and 0.1% copper sulphate. Where inhibition or stimulation of plant growth has resulted from the application of varying amounts of Photosensin, the effects on the plant have been attributed to the fluorescein content and not to any one or all of the other three compounds of the preparation. In the one case where Photosensin was not used, fluorescein was presumably employed with sodium bicarbonate to keep the high concentrations of fluorescein in solution. In addition, the experiments were carried out on a small

number of plants for each treatment where a synthetic substratum was used. Five plants per treatment are not sufficient to overcome individual variation in the case of such variable species as marigold and tomato when grown from seed.

The experiments reported here were designed to investigate further the effects of fluorescein, of Photosensin, and of the inorganic constituents of Photosensin on more extensive populations of a single variety of plant grown on a synthetic substratum.

Material and methods

Red kidney beans were grown under greenhouse conditions at two separate times, from October 9 to November 6, 1942; and from May 25 to June 14, 1943. In both cases growth was terminated when flower buds began to appear. The lot of seeds employed in the 1942 experiment proved irregular in germination, emergence, and development. The epicotyls of many seedlings did not elongate or continue development, although some plants produced shoots from buds in the axils of the cotyledons. Only those plants in which the epicotyl continued to develop were used, however, those with shoots produced from buds in the axils of cotyledons being discarded. A second lot of seed employed for the experiment of 1943 gave a very uniform stand of vigorous plants, of which the first simple leaves were exceptionally large.

Two plants were grown in 5 lb. each of pure quartz sand in glazed jars with side drainage at the bottom, the hole being covered with glass wool. Seeds

were planted four to a jar at 1-inch depth and immediately supplied with complete nutrient, the sand previously having been rinsed twice with distilled water. Just after the hypocotyl arch had straightened out, the seedlings were thinned to two per jar.

The nutrient medium consisted of 0.0060 M calcium nitrate, 0.0045 M magnesium sulphate, and 0.0045 M potassium-acid phosphate in distilled water. Microelements were supplied as 0.1 p.p.m. iron sulphate, 1.0 p.p.m. zinc chloride, 1.0 p.p.m. manganese sulphate, and 1.0 p.p.m. sodium tetraborate. No copper was added to the first series but was added as 1:50,000,000 copper sulphate in the second experiment to keep the medium comparable with that used by SELLEI *et al.* (7). All chemicals were of c.p. grade. The fluorescein was obtained from Eastman Company and the Photosensin was the commercial product of that name.

Hydrogen and hydroxylion determinations were made on the Coleman glass electrode electrometer no. 3. Osmotic pressures were determined according to the freezing-point depression method of LOOMIS and SHULL (4).

Harvesting was by rows of seven jars each, the tops being cut off at sand level and the roots rinsed free of sand in tap water. Both tops and roots were dried in a hot-air oven at 60°-70° C. for 18 hours. The dried samples were then placed in a desiccator over calcium chloride until constant weights were obtained. Weights were taken to the nearest hundredth of a gram.

The first experiment consisted of two treatments and a control. The concentrations of fluorescein were 1:50,000 and 1:500,000, the control being plain nutrient. There was some precipitation of the fluorescein at the 1:50,000 concen-

tration, but this was stirred up each time the solution was applied. The pH of the nutrient was 4.0 and was not affected by the addition of the fluorescein. Each treatment comprised 140 plants arranged in ten rows of seven jars each, the resulting thirty rows randomized on one bench in the greenhouse. Randomization was accomplished by assigning numbers to the treatments, shaking them in a container, and drawing them off until each row had been assigned the type of treatment. The plants were thinned to two per jar and treatment begun on October 15, 1942, 6 days after the seeds were placed in the sand. Solutions of the corresponding type were applied when the surface of the sand began to appear dry. This amounted to five applications of about 250 ml. each. The plants were harvested on November 6, 1942.

The five treatments (and a control) of the second experiment, the plants randomized as in the first experiment, were designed to observe the effect of a concentration of fluorescein of 1:10,000,000 and the accompanying amounts of inorganic compounds found in the quantity of Photosensin required to obtain this concentration of fluorescein. Each treatment and control consisted of 140 plants, following the method of the first experiment. A concentration of fluorescein of 1:10,000,000 was chosen, since the data of SELLEI (7) indicate this to be near the optimum. The treatment consisted of the following concentrations in nutrient solution: fluorescein 1:10,000,000; sodium bicarbonate 18.8:10,000,000; iron sulphate 0.2:10,000,000; copper sulphate 0.02:10,000,000; Photosensin to make 1:10,000,000 fluorescein.

The pH of the five dilute solutions of the second experiment was taken before and after addition of the substances. No

changes were observed. Four of the solutions were pH 4.0, the one with sodium bicarbonate being pH 3.95. Thinning of the plants and application of the treatments was done in May, 1943. The longer growing period required seven applications of about 250 ml. Harvesting occupied 3 days. All tops were cut on June 14, 1943. The roots were harvested on the two succeeding days, one bench on each day.

A third experiment was run on a smaller scale to observe the effects of considerable amounts of sodium bicarbonate over a range of concentrations with and without fluorescein, as this compound is the largest (93.9%) constituent of Photosensin. The sodium bicarbonate and fluorescein concentrations per liter of nutrient, together with the pH, were as follows:

Sodium bicarbonate	fluorescein, pH
56.4 gm., without	pH 8.40
56.4 with 3 gm.	pH 7.90
18.8 without	pH 8.40
18.8 with 1 gm.	pH 7.95
9.4 without	pH 8.30
9.4 with 0.5 gm.	pH 8.20
4.7 without	pH 8.05
4.7 with 0.25 gm.	pH 8.75

The solutions were applied to two jars of two to four plants for each treatment. The osmotic pressure of the solution containing 56.4 gm. of sodium bicarbonate per liter of nutrient was 24.85 atmospheres; that of the same amount of sodium bicarbonate plus 3 gm. of fluorescein was 21.39 atmospheres. No weights were taken on the plants of this experiment, since they were killed by the three highest concentrations of sodium bicarbonate and of it plus fluorescein. One application of each treatment was sufficient to produce the effects noted.

Results

In the first experiment employing only fluorescein, no consistent differences were noticeable among the living plants. Some irregularities occurred, but these could not be related to any treatment. Table 1 lists the dry weights of roots and tops, each weight representing the tops or roots of fourteen plants, to-

TABLE 1

FLUORESCIN IN RELATION TO GROWTH OF RED KIDNEY BEANS. DATA IN GRAMS DRY WEIGHT OF FOURTEEN PLANTS

CONTROL (PLAIN NUTRIENT)		FLUORESCIN IN NUTRIENT SOLUTION			
		1:50,000		1:500,000	
		Tops	Roots	Tops	Roots
12.54	3.90	12.66	1.04	13.54	2.30
14.60	3.12	14.68	1.97	18.87	2.84
13.56	3.04	12.20	1.55	12.31	3.14
14.13	3.51	15.48	3.80	9.31	2.83
11.91	2.95	16.16	5.94	14.25	5.94
11.22	3.67	14.91	3.84	12.67	5.02
12.36	2.50	11.89	5.43	12.53	4.72
13.91	3.57	13.39	5.35	10.57	5.70
13.06	4.57	14.58	4.60	9.01	2.52
16.94	4.94	11.94	4.97	12.15	4.44
134.23	35.57	137.89	38.49	118.21	39.45
Total..	169.80	Total..	176.38	Total..	157.66

gether with the total weights of tops and roots for each treatment. The weight of the plants treated with the 1:500,000 dilution is 12.14 gm. less than the control, and the plants treated with the 1:50,000 dilution weigh 6.58 gm. more than the control. On a percentage basis, the plants of 1:50,000 dilution weigh 3.8% greater, and those treated with the 1:500,000 dilution weigh 6.2% less, than the controls.

In comparing the data in tables 1 and 2, greater regularity in weights are shown in table 2, indicating an even

more uniform stand of plants. Likewise, no visible differences were observed in the growing plants of the second experiment. The copper-sulphate treated plants of the second experiment weighed the most, with the fluorescein- and Photosensin-treated plants second and third highest in weight, respectively. The plants of the control and those treated with iron sulphate and sodium bicar-

were killed. All plants receiving fluorescein plus sodium bicarbonate and sodium bicarbonate alone in the three highest concentrations were dead 1 day after treatment. Those receiving the lowest concentrations showed drying at the edges of the oldest leaves at the end of 2 days, when the experiment was terminated. The plants were of the same stage of development as were those of the first

TABLE 2

GROWTH OF RED KIDNEY BEANS IN RELATION TO FLUORESCEIN, PHOTOSENSIN, AND INORGANIC CONSTITUENTS OF PHOTOSENSIN. CONCENTRATIONS AS INDICATED IN NUTRIENT SOLUTION. DATA IN GRAMS DRY WEIGHT OF FOURTEEN PLANTS

CONTROL (PLAIN NUTRIENT)		0.2:10,000,000 IRON SULPHATE		1:10,000,000 FLUORESCEIN		18.8:10,000,000 SODIUM BICAR- BONATE		0.02:10,000,000 COPPER SULPHATE		PHOTOSENSIN TO MAKE 1:10,000,000 PHOTOSENSIN	
Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
20.61	5.13	21.28	4.17	22.07	4.60	21.93	4.74	23.87	5.35	20.47	4.95
23.31	7.07	23.20	6.19	22.29	5.20	22.59	4.65	23.30	4.51	23.73	6.03
22.96	5.28	23.38	5.63	23.23	4.71	20.27	6.78	23.65	5.14	23.14	4.68
18.47	4.95	21.56	4.45	21.73	4.64	21.33	4.44	23.43	5.88	23.75	5.38
24.29	5.15	23.26	4.85	21.93	4.35	20.90	5.02	24.10	5.14	21.82	4.12
20.75	5.19	22.04	4.56	22.60	4.40	22.54	4.54	22.71	4.81	22.14	4.16
20.95	4.56	21.99	4.63	25.19	5.95	23.77	4.75	22.48	4.29	21.54	4.79
22.98	4.95	22.49	4.26	23.33	4.55	21.50	4.04	23.73	5.65	22.30	4.93
21.51	4.46	21.81	4.81	22.51	4.47	22.74	4.64	21.12	4.19	21.97	5.00
21.21	5.17	20.95	4.82	22.20	5.28	22.55	5.00	22.18	5.17	23.15	5.72
217.04	51.91	221.96	48.37	227.08	48.15	220.12	48.60	230.57	50.13	224.01	49.76
Total.. 268.95		Total.. 270.33		Total.. 275.23		Total.. 268.72		Total.. 280.70		Total.. 273.77	

bonate were all very nearly the same weight.

The sodium-bicarbonate treatments of the third experiment with and without fluorescein in the three highest concentrations killed the plants. The highest concentration of sodium bicarbonate alone, and with the 3 gm. of fluorescein, produced noticeable effects on the plants within 5 minutes after application. At the end of 15 minutes the plants had collapsed. Fluorescein began to show in the veins within half an hour after treatment, indicating that the root systems

and second experiments when treatments began; that is, the hypocotyl arch had just straightened out.

Discussion

In considering the data presented by SELLEI *et al.* (7) for the sand culture experiments consisting of five plants per treatment, a lack of consistency over the range of treatments is apparent. Although most of the treated plants developed higher weights than the controls, these in several instances are higher than the treated plants. Furthermore,

the series of treatments do not form a smooth curve of increasing weights with decreasing concentrations, or vice versa. Nor can the effect on the plants logically be attributed to the fluorescein in the Photosensin preparation employed by them. As was pointed out earlier in this paper, Photosensin is composed of four compounds, three inorganic ones and the organic fluorescein. The inorganic compounds have cations definitely known to have effects on the growth of plants, the anions not being important in the concentrations used. Likewise, the stunting effect attributed to the fluorescein in high concentrations cannot be divorced from the inorganic compounds of the Photosensin preparation.

The lack of pronounced visible differences among the living plants and the small differences in dry weights shown in tables 1 and 2 indicate that fluorescein by itself in the three concentrations employed is without appreciable effect on the growth of red kidney beans. If the slight additional weight made by the plants treated with the 1:50,000 fluorescein concentration was attributable to the dye, then the slightly less weight made by the plants receiving 1:500,000 and 1:10,000,000 fluorescein cannot easily be explained as an actual inhibition of growth. Rather, the small differences in dry weights and appearance of the living plants indicate that these are within the variations of the populations of plants employed.

The data supplied by the second experiment do not appear to show significant differences, inasmuch as the greatest weight was made by those plants receiving the slight additional copper found at a concentration of fluorescein of 1:10,000,000 as Photosensin. In all probability, the variations in weights are

those to be expected with populations of this size under ordinary conditions.

Sodium, which is in dispute as an essential element for plants, is not without effect on plants and cannot be ignored when used in low concentrations as sodium bicarbonate in the Photosensin preparation. MULLISON and MULLISON (5), working with barley, found evidence of substitution for potassium at almost all levels of potassium and sodium.

Any further experimentation to observe the effects of fluorescein as a stimulator of plant growth must take into account that Photosensin is a preparation consisting of fluorescein, sodium bicarbonate, iron sulphate, and copper sulphate. If the effects are to be referred to fluorescein, this dye must be employed by itself.

Those plants receiving the high concentration of sodium bicarbonate and sodium bicarbonate-fluorescein in nutrient solution were killed by the treatments: The effects on the plants cannot be called merely stunting of growth, for death ensued within a matter of minutes after the plants had been subjected to the treatments. The killing is about as rapid when the sodium bicarbonate-fluorescein solutions are used as when the sodium bicarbonate alone is applied. Although the pH values are relatively alkaline for good growth, the rapidity with which these plants were killed points rather to the high osmotic pressures (24.85 atmospheres for the highest concentration of sodium bicarbonate) to which the roots were subjected. The precipitation of much of the magnesium and calcium in the form of carbonates by the extensive amounts of sodium bicarbonate removes the buffering qualities of these metallic ions from the solu-

tion. Likewise, the nitrate and sulphate ions released by these precipitated cations allow for more thorough ionization of the sodium in solution. All these factors probably contribute to the rapidity with which the plants are killed, but it would appear that the high osmotic pressures are largely responsible.

HAYWARD and LONG (3) and GERNER (1), working with high osmotic pressures attained by sodium sulphate and sodium chloride in nutrient solution, found that killing was common in the higher concentrations of these salts. HAYWARD and LONG report that killing was complete for tomato at an osmotic pressure of 6 atmospheres attained by the addition of sodium sulphate to the nutrient solution, whereas GERNER reports killing as very frequent for tomato at the highest concentrations, between 11 and 12 atmospheres. In both papers it is stated that the high osmotic values were attained by gradually increasing the amounts of solutes over a period of days. HAYWARD and LONG do not report a sodium toxicity as such, but they do note that "it seems possible, however, that the sodium ion may have an inhibitory effect on growth."

Thus, it can be assumed that the rapid change in the physico-chemical envi-

ronment of the root systems of the plants subjected to the extensive amounts of sodium bicarbonate caused death of the plants, and the observed effects cannot be attributed to the fluorescein when it is kept in solution by sodium bicarbonate.

Summary

1. Red kidney beans were grown in pure quartz sand with nutrient solution to which were added in separate series Photosensin and the constituents of Photosensin—fluorescein, iron sulphate, sodium bicarbonate, and copper sulphate.

2. There were no pronounced visible differences among the living plants, and the differences in dry weight were small. Those plants receiving the small amount of copper sulphate weighed the most.

3. Apparently fluorescein does not stimulate growth of red kidney beans.

4. The so-called stunting effect heretofore ascribed to the high concentrations of fluorescein is shown to be actual killing by the extensive amounts of sodium bicarbonate used to keep the fluorescein in solution.

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CURRENT LITERATURE

John Merle Coulter: Missionary in Science. By ANDREW DENNY RODGERS III. Princeton, New Jersey: Princeton University Press, 1944. Pp. 321.

This is not a biography in the usual sense of the term, but rather a constructive and illuminating contribution to the history of botany in the United States through the life-span of one individual (1851-1928). It traces his influence, in collaboration with his contemporaries, upon the course and development of botanical science—particularly systematics and morphology—during that period. The discussion and territory covered range far, and as first one individual and then another is discussed, the central figure—Professor JOHN MERLE COULTER—often becomes obscured, although the theme is maintained. The names of ARTHUR, BAILEY, BESSEY, BRITTON, COVILLE, COWLES, FARLOW, GRAY, MACDOUGAL, ROSE, and a score of others cross the pages with such frequency, and are often treated at such great length, that touch with the personality of Dr. COULTER is all but lost until near the end of the book. But despite these complications, the attentive reader readily weaves it all into a clear, vivid history of an unusually active, formative period of botanical development.

The characterization of Dr. COULTER which eventually emerges differs in many details from that seemingly carried by a large body of his students. The author paints him, not simply as a great teacher, editor, and scientist aloof from application and practicality, but—through quotations and by other means—as emphasizing early in his career and again and again later the practical values and economies to be derived from the application of the principles of pure science. His broad interests in the development of the fields of physiology, ecology, and later those of pathology and genetics are stressed. Dr. COULTER's contributions through lectures and writings to the field of secondary and advanced education are detailed at length. The reasons and events which led to the broadening of his earlier interests in systematics to include morphology and other phases of botanical endeavor are pointed out. Emphasis is placed on the fact that Dr. COULTER's deeply religious interests never wavered, and on the fact that he found no disharmony between evolutionary and Christian doctrines. Dr. COULTER stressed the relation and application of science to agriculture and to every-day life. Some of his statements made nearly half a century ago are as pertinent, clear, and direct as many which today pass as being strictly modern, daring, and revolutionary!

Despite its trying style, the work has been pre-

pared with great attention to accurate detail. Material from letters, lectures, articles, and other contributions has been extensively drawn upon, and members of Dr. COULTER's family and many of those who had worked with him, either as colleagues or students, were interviewed directly and at length.

As a report on an era of development of botanical science in the United States, the book should occupy a place on the "must" list of botanical reading and study of every individual who desires a clear picture of this science.—E. J. KRAUS.

Mitosis: The Movement of Chromosomes in Cell Division. By FRANZ SCHROEDER. New York: Columbia University Press, 1944. Pp. x+110. Illustrated, \$2.00.

This treatise is concerned with a subject to which the author has devoted many years of intensive and fruitful research. It presents a résumé and a critical review of the various hypotheses advanced to account for the mitotic process. In the main, only investigations of the last 20 years are considered in detail, the older being included only when necessary to provide a background. The author discusses the structure of living and fixed cells, the actuality of structural elements, the nature and origin of the spindle apparatus and the hypotheses of mitosis (including contraction, expansion, and their variations), viscosity and hydration, electrostatics, diffusion, streaming, hydrodynamics, tactoids and chromosome autonomy. These topics are presented fairly and without prejudice, with the evidence for and against each clearly brought out. The need for further critical research in this field is forcefully indicated.—J. M. BEAL.

Bibliography of References to the Literature on the Minor Elements and Their Relation to Plant and Animal Nutrition. Fifth Supplement to Third Edition. New York: Chilean Nitrate Educational Bureau, Inc., 1944. Pp. 96.

The fifth supplement contains approximately 700 abstracts, dealing with 117 plants or plant groups and with 48 elements, excluding those treated in the few abstracts of the rare earth and miscellaneous sections at the end. The abstracts are arranged in alphabetical order of the elements. The three indices of the previous supplements—element, botanical, and author—are continued, and a fourth—animal nutrition—is added. These indices add much to the value of the book.—S. V. EATON.

EFFECTS OF ISOSMOTIC CONCENTRATIONS OF INORGANIC AND ORGANIC SUBSTRATES ON ENTRY OF WATER INTO CORN ROOTS

H. E. HAYWARD AND WINIFRED B. SPURR¹

Introduction

The entry of water into plant roots is conditioned by several factors. The metabolic status of the root with respect to carbohydrate reserves, the biochemical changes incident to the entry of solutes from the soil solution, the anatomical differences in roots due to differential rates of growth, and the specific or genetic differences in roots—these all operate to accelerate or retard intake of water (5, 6, 22). Soil factors which influence rate of entry of water include the water content of the soil, soil aeration, and the amount of salt in the soil solution (23). The force against which the plant roots must act to absorb water is conditioned in part by the osmotic pressure of the solution and by the "capillary" forces of the soil particles (1).

Studies of the salt tolerance of a number of plants in sand and water cultures indicate that, in general, the osmotic pressure of the substrate is the most important factor in restricting growth and development under saline conditions. It has also been shown that the toxic effect of specific ions operates in this connection.

Most of the quantitative studies of water intake by intact roots have been made with substrates of relatively low

osmotic pressure—tap water or a nutrient culture solution. GREGORY and WOODFORD (4) report a few preliminary experiments on water intake which showed extreme variability. HÖHN (9), SIERP and BREWIG (19), ROSENE (17), and HAYWARD, BLAIR, and SKALING (5) have made quantitative studies which indicate that maximum rates of water intake occur in the basal regions of young roots, 60–100 mm. from the root tip, rather than in the apical zone immediately above the root cap.

A few studies have reported quantitative data on the rate of water intake by roots as affected by the osmotic pressure of the substrate. ROSENE (18), using attached roots of *Allium cepa*, found that substrates with osmotic pressures of 4.2–5.7 atmospheres "represented the range of critical concentrations of sucrose and KNO₃ which will balance the internal factors that operate to determine the velocity of water transport across the epidermal boundary of local root regions." At 25°C. and 100% relative humidity, an osmotic pressure of 5.14 atm. stopped absorption in the majority of intact roots. With leaves exposed to constant light and air at 50% relative humidity and 25°C., absorption ceased when the osmotic pressure of the substrate was 6.5 atm.

TAGAWA (20), using intact seedlings of *Phaseolus vulgaris*, studied intake of water in substrates of various osmotic pressures. Using sucrose, Knop's solu-

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tion, CaCl_2 , KCl , and mixtures of the two salts at osmotic pressures ranging from 0.03 to 2.6 atm., he found that the high pressures reduced intake to about 40% of the rate in distilled water. At equal osmotic pressures (2.4 atm.) intake was about the same with sucrose, KCl , and CaCl_2 , and somewhat higher with Knop's solution. In mixtures of the chloride salts he found that K^+ accelerated and Ca^{++} retarded entry of water.

HAYWARD and SPURR (6) used NaCl added to a nutrient solution to produce osmotic pressures of 2.8 and 4.8 atm. As compared with roots in a control substrate (0.8 atm.), the decrease in rate in the zone of most rapid intake was 64% in the 2.8 atm. and 82% in the 4.8 atm. substrate.

It has been stated that water is equally available to the plant roots from field capacity to the permanent wilting percentage, 0–15 atm. of soil moisture tension (7, 21). Since the osmotic pressure of the substrate is an important factor in determining the rate of water entry into roots, more quantitative data are needed to show to what extent specific ions or salts commonly occurring in the soil solution in saline areas influence the entry and at what pressure there is cessation of intake. In these studies, NaCl , Na_2SO_4 , and CaCl_2 were used as single salts added to the nutrient solution; and, to avoid the possibility of toxic action due to salts, solutions of mannitol and sucrose isosmotic with those of saline substrates were also tested.

Procedure

The potometric device used has been described (5). It can be adjusted at any point on the root except where laterals diverge and is adapted to use in substrates of high osmotic pressure, since the test root is submerged in a large

volume of solution (33 liters), which eliminates the possibility of significant changes in osmotic pressure due to the intake of solutes. The tests were run under constant conditions of temperature, $72^\circ\text{F.} \pm 1^\circ$, and $70\% \pm 5\%$ relative humidity. The light was supplied by overhead illumination with Westinghouse white fluorescent tubes. The solutions were continuously aerated with carbon-pipe aerators.

As in earlier studies (5, 6), Mexican June corn was used. The test plants were grown in the greenhouse in nutrient solution until they attained the desired size, 10–12 inches tall with five or six expanded leaves and a cycle of adventitious roots exceeding 12 cm. in length. Potometers were attached at root levels 6 and 10 cm. from the root tip, since the 4-cm. zone between these levels has been shown to be the region of most rapid water intake (6). Each trial was run for $5\frac{1}{2}$ or 6 hours, with potometric readings at hourly or half-hourly intervals—depending upon the rate of entry. The first readings were not made until the root had been in the substrate for a minimum of 30 minutes, to avoid possible transference reactions (2, 15, 20) or initial ionic effects (13).

In addition to the base nutrient solution (0.8 atm. osmotic pressure) identical with that in which the plants were grown, saline substrates of two higher osmotic pressures were used (2.8 and 4.8 atm.). The tests with organic solutions were run at 0.8, 2.8, and 4.8 atm. with no added nutrient salts. A few trials were made with a sucrose substrate at 6.8 atm. The freezing-point depression method was employed to determine the osmotic pressures of the substrates, and variations from the theoretical value did not exceed 0.2 atm. in any trial.

Investigation

INORGANIC SUBSTRATES

The rates of entry of water for intact roots in inorganic substrates and those obtained with roots in the base nutrient solution are shown in table 1 and figure 1. The rate of intake was slightly higher at the 10-cm. root level than at the 6-cm. level in all cases except when CaCl_2 was the added salt; but the differences in rate at the two levels are significant only in the control substrate. Differences in rate of intake in the three saline substrates at isosmotic pressures are not significant at either the 2.8- or 4.8-atm. levels. This is true when the comparisons are made between rates at either root level (6 or 10 cm.) or between the average mean rates of the two levels.

In all three saline solutions, the difference in rate of water intake between substrates at 0.8, 2.8, and 4.8 atm. osmotic pressure was highly significant when the average mean rate of the individual roots was used as a basis of comparison. At the 6-cm. level there was less difference in rate between the base nutrient and the saline substrates at 2.8 atm. than at the 10-cm. level. In the latter, all differences were highly significant; but at the 6-cm. level, differences in rate between the base nutrient and the CaCl_2 substrates at 2.8 atm. were not significant.

ORGANIC SUBSTRATES

To eliminate the possibility of toxic effects due to salts on the rate of entry of water, experiments were set up using mannitol and sucrose as the solutes. In both cases the substrates were adjusted to 0.8, 2.8, and 4.8 atm. osmotic pressure, and an additional one was run with sucrose at 6.8 atm. to determine the approximate osmotic pressure at which in-

take of water would stop under the experimental conditions of these studies.

The rates of water intake for intact roots in organic substrates are given in table 2 and figure 2. In all cases the rate was greater at the 10-cm. level than the 6-cm. level; but the differences were not significant, except at 0.8 atm. osmotic pressure, in which results for the organic substrates agreed closely with those of the base nutrient solution. Using the average of the mean water intake for the individual roots, highly significant differences were found in the rates at 0.8, 2.8, and 4.8 atm. osmotic pressure, regardless of the organic substrate used. A comparison of the rates of entry for mannitol and sucrose at isosmotic pressures shows no significant differences in water intake at 0.8 and 4.8 atm. but a significant difference at 2.8 atm. No explanation is advanced for this exception to the relationships determined with all other substrates at this osmotic pressure, and for all substrates, including sucrose at the 0.8- and 4.8-atm. levels.

A few roots were tested in a sucrose solution at 6.8 atm. osmotic pressure. No intake was observed; in fact, a very slight outward movement was noted. Since this result indicated that the critical concentration which would stop intake of water under these experimental conditions was an osmotic pressure of slightly less than 6.8 atm., the pressure of the sap of corn roots grown in the base nutrient solution was determined by a method² suggested by HOAGLAND (8). A value of 5.7 atm. was obtained using sap from roots taken in the morn-

² Roots rinsed quickly in distilled water, centrifuged at a r.p.m. to produce a force 40-45 \times gravity, frozen rapidly at -15°C ., thawed, the sap expressed at 8000 pounds pressure per sq. inch, and freezing-point depression determined.

TABLE 1
MEAN RATE OF ENTRY OF WATER FOR ROOTS IN BASE NUTRIENT AND
INORGANIC SUBSTRATES EXPRESSED AS $\text{MM}^3/\text{MM}^2/\text{HR.}$

ROOT LEVEL (DIS- TANCE FROM ROOT TIP)	SUBSTRATES AND OSMOTIC PRESSURE						
	Base nutrient	B.n. + Na_2SO_4		B.n. + NaCl		B.n. + CaCl_2	
	0.8 atm.	2.8 atm.	4.8 atm.	2.8 atm.	4.8 atm.	2.8 atm.	4.8 atm.
10 cm.	0.243 (30)*	0.140 (16)	0.052 (19)	0.134 (26)	0.044 (18)	0.118 (28)	0.041 (17)
6 cm.	0.164 (30)	0.130 (18)	0.037 (18)	0.115 (28)	0.039 (20)	0.134 (26)	0.033 (17)
Av. 10 cm. + 6 cm. †	0.204 (60)	0.135 (32)	0.042 (34)	0.123 (52)	0.041 (34)	0.127 (52)	0.036 (32)

* Figures in parentheses indicate number of tests run. In some cases, the number shown for the average rate is less than the sum of the tests for the two root levels, since only paired observations were used in computing average rate and occasionally one of the pairs is missing.

† Average of mean water intake for individual roots.

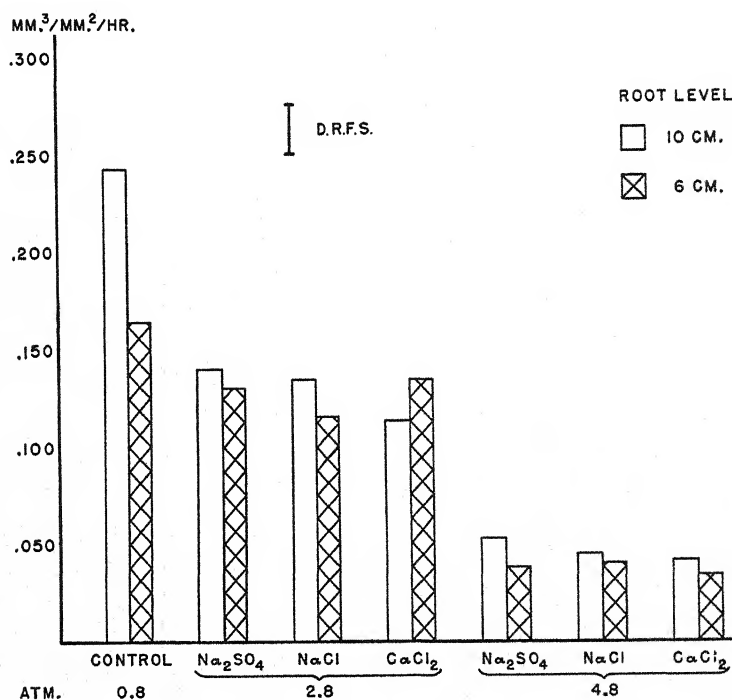


FIG. 1.—Effect of inorganic substrates on rate of entry of water into adventitious roots of corn at 10- and 6-cm. levels. Sodium sulphate, sodium chloride, and calcium chloride added to base nutrient solution (0.8 atm.) as single salts to produce isosmotic pressures of 2.8 and 4.8 atm. Difference required for significance (D.R.F.S.) as derived from pooled error variance = 0.0263 at 5% level.

TABLE 2

MEAN RATE OF ENTRY OF WATER FOR ROOTS IN BASE NUTRIENT AND ORGANIC SUBSTRATES EXPRESSED AS $\text{MM.}^3/\text{MM.}^2/\text{HR.}$

SUBSTRATES AND OSMOTIC PRESSURE								
ROOT LEVEL (DISTANCE FROM ROOT TIP)	Base nutrient	Mannitol			Sucrose			
		0.8 atm.	0.8 atm.	2.8 atm.	4.8 atm.	0.8 atm.	2.8 atm.	4.8 atm.
	10 cm....	0.243 (30)*	0.257 (8)	0.123 (15)	0.057 (8)	0.255 (12)	0.101 (18)	0.045 (9)
6 cm....	0.164 (30)	0.157 (8)	0.116 (14)	0.030 (7)	0.174 (10)	0.075 (20)	0.035 (9)	0.001 (3)
Av. 10 cm.+6 cm.†.	0.204 (60)	0.207 (16)	0.122 (28)	0.042 (14)	0.218 (20)	0.089 (36)	0.040 (18)	-0.001 (6)

* Figures in parentheses indicate number of roots tested. In some cases, the number shown for the average rate is less than the sum of the tests for the two root levels, since only paired observations were used in computing average rate and occasionally one of the pairs is missing.

† Average of mean water intake for individual roots.

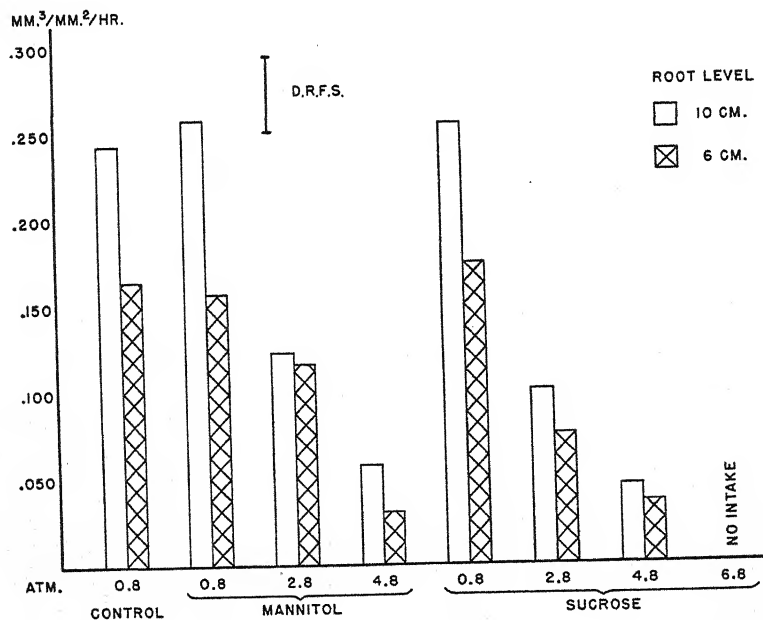


FIG. 2.—Effect of organic substrates on rate of entry of water into adventitious roots of corn at 10- and 6-cm. root levels. Mannitol and sucrose added to tap water to produce desired osmotic pressures. Difference required for significance (D.R.F.S.) as derived from pooled error variance = 0.0452 at 5% level.

ing—the time the experiments were regularly started.

COMPARISON OF INORGANIC AND ORGANIC SUBSTRATES

The average mean rates of water intake for individual roots in inorganic and organic substrates are shown in figure 3. In general, these data indicate no significant difference in rate of intake at a given

of osmotic pressure were highly significant. Considering the rate of entry at the 0.8 level as 100%, the reduction in rate at 2.8 atm. ranged from 34% with Na_2SO_4 substrate to 59% with the sucrose. Excluding sucrose, the average reduction for the other four substrates was 38%. At the 4.8-atm. level, the reduction varied from 79% with Na_2SO_4 to 82% with CaCl_2 and sucrose, with an average

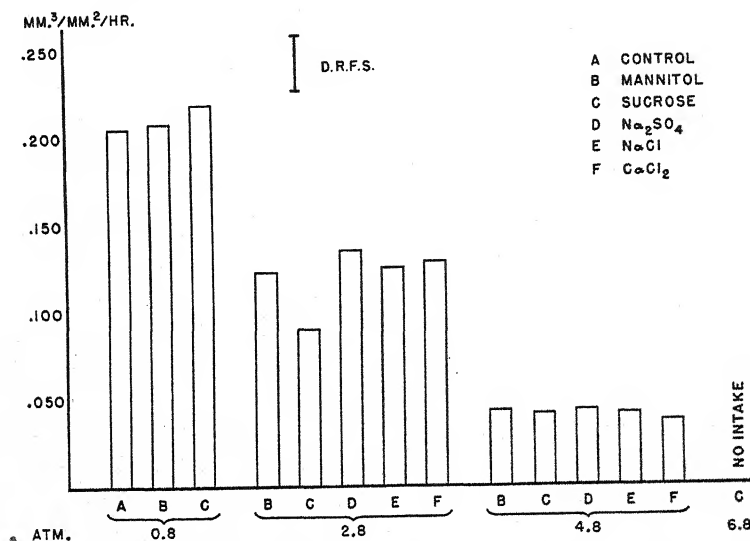


FIG. 3.—Comparison of average mean rate of entry of water for individual roots in organic and inorganic substrates at isosmotic pressures. Difference required for significance (D.R.F.S.) as derived from pooled error variance = 0.0327 at 5% level.

osmotic pressure of the substrate, regardless of the solute used. At the 0.8-atm. level, the rates for the organic substrates were slightly higher than for the control solution containing nutrient salts. As already noted, an exception occurred at the 2.8-atm. level, where the intake for sucrose was significantly lower than the rates of the other four substrates. Very close agreement in rate was obtained with roots tested in five substrates at 4.8 atm. (fig. 3).

In all substrates, differences in average mean intake between any two levels

reduction of 81% for the five substrates tested.

Discussion

The data indicate that total osmotic pressure of the substrate, regardless of the solute involved, is a primary factor limiting entry of water into roots. When uniform corn plants grown under the same nutritional and environmental conditions are transferred to substrates whose osmotic pressures range from 0.8 to 4.8 atm., there is marked reduction in rate of intake with increasing pressure. In

general, the reduction at a given osmotic pressure does not vary significantly due to the kind of solute used. When compared with the 0.8-atm. substrates, the reduction in rate of intake at 4.8 atm. varied only 3% (79–82%), regardless of whether the solute was organic (sucrose or mannitol) or a single salt (Na_2SO_4 , NaCl , or CaCl_2).

Results in essential agreement with these data have been reported by ROSENE (18), who found that the same concentrations of sucrose and KNO_3 would inhibit water transport across the epidermal boundary in *Allium cepa*. TAGAWA (20), experimenting with intact seedlings of *Phaseolus vulgaris* in substrates of lower osmotic pressure (approximately 2.4 atm.) than those reported here, found little difference in absorption with isosmotic solutions of sucrose, CaCl_2 , or KCl . He did, however, obtain marked differences in absorption when mixtures of CaCl_2 and KCl were used in ratios of 8:2 and 2:8, respectively. Increase in K^+ accelerated absorption, while increase in Ca^{++} retarded water intake.

It might be assumed that the difference in rate of water entry with mannitol and sucrose substrates is related to a differential permeability of the absorbing cells to these solutes. However, COLLANDER and BÄRLUND (3), studying the uptake of sugar by *Chara* from dilute and more concentrated solutions (0.25–6.15 atm.), found that no appreciable amounts of sucrose, glucose, or mannitol were taken up by *Chara* cells in 48 hours. Using approach-grafted tomatoes to study the intake of water and nutrients by roots, LONG (11) found that sucrose reduced water intake approximately 30% more than NaCl at equal osmotic pressures (4.8 atm.). He attributed part of the difference to a greater reduction in the pressure of the NaCl solution

(4.15 atm.) than the sucrose substrate (4.5 atm.) during the course of the experimental run, but he pointed out that in absolute amounts more sugar than NaCl was removed by the roots. LONG suggests that the absorbed sugar, being respirable, might "contribute less to the internal osmotic pressure than the salt which remains osmotically active." His results are in line with our data for the experiments run with substrates at 2.8 atm. osmotic pressure but are at variance with those at 4.8 atm., where the difference in rate of intake between the sucrose and mannitol substrates was only 5% and not statistically significant.

The effect of various ions on permeability has been investigated extensively. OSTERHOUT (14), using the conductivity method on the tissue of *Laminaria*, found that Na^+ and K^+ increased permeability regardless of the anion with which the cation was paired, while Ca^{++} tended to decrease it. LUCKÉ (12) states that decrease in permeability is produced by cations which act in proportion to their valence. On this basis it might be expected that intake of water would be more rapid with a NaCl substrate than with an isosmotic solution of CaCl_2 . Our data indicate no significant differences with the salts used. This may be related to the observation of OSTERHOUT (13) that the inhibiting effect of CaCl_2 is not permanent and if exposure is prolonged will be followed by an increase in permeability. Using *Laminaria*, he found that the peak in resistance was reached in 15 minutes and was maintained for about an hour, after which there was a rapid drop. Since our readings were not begun until the plants had been in the substrate for at least 30 minutes, it seems probable that any initial effect of the Ca^{++} ion would not be significant. In fact, examination of the first half-hourly

readings as compared with the rates of intake at subsequent half-hour intervals does not reveal any consistent trend with the CaCl_2 substrate. TAGAWA (20) found the "transference" reaction to be about the same for sucrose, KCl, and CaCl_2 solutions. When intact bean seedlings were placed in the concentrated substrates (2.4 atm.) there was a sudden drop in water absorption, which lasted about 30 minutes, and then intake increased gradually—reaching constant values in at least one hour after transference.

The data obtained by us as to the osmotic pressure at which intake of water ceases are in general agreement with those of ROSENE (18), who found it to be 6.5 atm. for *Allium cepa* when the leaves were exposed to constant light and air at 50% relative humidity and 25° C. Our value of approximately 6.8 was obtained under a constant temperature of 22° C. and relative humidity of 70%. KÖHNLEIN (10), using decapitated corn plants and a manometric technique, was able to stop exudation by adding sugar solution of 2.5 atm. osmotic pressure. This value is in line with our previously published results for decapitated corn plants, in which the rate of water intake was reduced 56% as compared with intact plants (6). On the basis of his critical concentration and our values as to percentage reduction in intake due to decapitation, it would probably require a substrate of approximately 5.7 atm. to inhibit entry of water.

These data have interesting implications in relation to practical problems of irrigation agriculture in saline areas. The range in the "capillary" force with which water is held by soil particles between field capacity and the permanent wilting point is approximately 0.1–15 atm. of

tension (16). If water intake by corn roots is reduced as much as 80% when the osmotic pressure of the substrate is raised from 0.8 to 4.8 atm., and completely inhibited at 6.8 atm., it is questionable whether water held in the soil with a force of 6.8 atm. is readily available to the plant. Since the moisture content corresponding to such a force lies between the moisture equivalent and the permanent wilting percentage, it would appear that water is not equally available between field capacity and the wilting range, as has been proposed by some investigators (7, 21). It therefore seems clear that both the force with which water is held by the soil particles and the amount of soluble salts present in the soil solution are important factors when considering the water available to the plant.

The salt tolerance of a plant may be related in part to the osmotic pressure of the sap that a given species can maintain. This, in turn, is undoubtedly conditioned by its nutritional status. Hence a more complete understanding of the metabolism of the plant as related to the synthesis of carbohydrate reserves, the effect of such reserves on concentration of the sap, and the relation of fertilizer practices to metabolism is indicated.

Summary

The data are interpreted as indicating that the osmotic pressure of the substrate is one of the primary factors in controlling the rate of entry of water into roots. It is also recognized that the character of the solute and toxicity of salts or ions may have a significant bearing on the function of water intake, and further experiments are planned—especially with mixed salts. The conflicting

data regarding the effect of organic substrates are discussed in the light of other work. The results with inorganic substrates indicate that, under the conditions of these experiments, no significant

differences in rate of intake occur when isosmotic pressures of Na_2SO_4 , NaCl , or CaCl_2 are used as single salts.

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CHANGES IN THE ORGANIC-ACID CONTENT OF VALENCIA ORANGES DURING DEVELOPMENT¹

WALTON B. SINCLAIR AND R. C. RAMSEY

Introduction

It is well known that the free acidity of orange juice decreases and the pH increases during growth and maturation of the fruit. These facts are of practical importance in establishing maturity tests for commercial purposes, but they do not reveal any information about the kinds and amounts of organic acids produced in the fruit with the advance of the season.

In previous studies, SINCLAIR, BARTHOLOMEW, and RAMSEY (9), working largely with mature citrus fruits, have shown that the total acidity of orange juice is due chiefly to citric and malic acids. The concentration of malic acid in different juices varied only slightly, however, as compared with the changes in the citric-acid content. With one exception, the samples studied had a malic-acid content of 1.40–1.77 mg. per milliliter of juice, while the citric-acid content varied from 8.38 to 25.39 mg. per milliliter. It is thus evident that variations in acidity of the orange are due chiefly to changes in the citric-acid concentration. A definite relation was also found between the free-acid—combined-acid balance and the pH of the juice. The concentration of the combined acids in the juice is remarkably uniform; this means that the free-acid concentration is the chief variable.

On the basis of these results, experiments were planned to study the changes in the organic-acid constituents of the fruit of Valencia orange trees during

ripening. Such experiments required sampling of the fruit from a given plot of trees at intervals during the growing season and subsequent determination of the pH, the soluble-solids content, the citric- and malic-acid contents, the potentiometric titration curve, and the alkalinity of the ash of the juice of each sample. The size and weight of the fruit, dry matter of the pulp, and the total free acid per fruit were also determined on each sample. From such determinations, the relation of pH to the free acid, and also to the free-acid—combined-acid balance, was studied. The results of these investigations are reported here.

Material and methods

Samples of fruit for this experiment were taken at approximately monthly intervals during the season from October 2, 1943, to May 2, 1944, from a plot of nine Valencia orange trees selected for this study. At the time of the monthly sampling, six average-sized fruits were picked at intervals around the circumference of each tree. The equatorial and longitudinal axes were measured on the fifty-four fruits in each sample, for the estimation of average size and surface area of the fruit. Twenty fruits were taken at random from the sample and weighed, first with and then without peel. Of the twenty peeled fruits, four of average weight were ground in a Waring blender, strained, and made to volume; an aliquot was then titrated to determine the total free acidity per fruit. To determine dry weight, slices of the pulp from the peeled fruits were placed

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in aluminum weighing cans and dried in a vacuum oven for 60 hours at 65°C.

The remaining thirty-four fruits of each sample were juiced for the quantitative determinations of the acid constituents of the juice. For these analyses about 300 ml. of centrifuged juice was used. The refractive index was determined with an Abbé refractometer at 27°C. The percentage of soluble solids and the specific gravity were read from a sucrose table. All pH values were determined with a Beckman glass-electrode pH meter at 23°C. For the titrations and the citric-acid analysis, the juice was diluted with distilled water so that 10 ml. was equivalent to 1 ml. of juice.

The free acidity was determined by titrating an aliquot portion of the juice with standard NaOH to the phenolphthalein end point. The inflection point on the (potentiometric) titration curve was taken to represent most accurately the amount of free acidity in the juice. The quantitative determination of citric acid, free and combined, was made on the juice samples by the pentabromacetone method of PUCHER *et al.* (8). Since their procedure specifies a quantity of citric acid of 1 to 20 mg., it was necessary to modify their method slightly when juice samples containing over 20 mg. citric acid per milliliter were being studied. The only change necessary was an increase in the amount of standard AgNO₃ added in the final steps to measure the bromide ion present.

In the determination of the malic acid present, it was necessary to separate the organic acids from the other constituents in the juice. HARTMANN and HILLIG'S (6) method was used for extracting these acids. Ten milliliters of saturated lead acetate was added to 25 ml. of undiluted juice, and the mixture was diluted to 135 ml. with 95% ethyl alcohol. The

precipitated lead salts of the organic acids were separated from the solution by centrifuging and were then suspended in approximately 200 ml. of H₂O and heated to boiling. Hydrogen sulphide was bubbled through the mixture until it was saturated, and the lead sulphide then filtered off. The filtrate containing the organic acids was made to a volume of 250 ml., of which 10 ml. was equivalent to 1 ml. juice. The malic acid was determined quantitatively on 10 ml. of this solution by the method of PUCHER *et al.* (8).

The amount of combined organic acid in the juice was found experimentally by measuring the alkalinity of the ash from an aliquot of juice. For each sampling, four 50-ml. aliquots of juice were ashed at 450°C. to a white residue. To this ash was added 5 ml. of standard 2 N HCl, and the whole was washed into a 100-ml. volumetric flask. The flask was made to volume with water, and a 10-ml. aliquot was titrated with standard NaOH. The difference in titer of NaOH between the sample and a blank solution of the HCl represents the alkalinity of the ash. The amount of HCl (in milliequivalents) neutralized by the ash is a close approximation of the milliequivalents of combined organic acid in the juice, since these organic salts decompose to carbonates during the ashing.

Results

CHANGES IN FREE-ACID CONTENT WITH FRUIT GROWTH

The components of fruits are usually expressed as percentages of the fresh or dry weight. Percentages calculated on these terms are satisfactory for most practical purposes, but from a physiological standpoint it is sometimes important to know the amount of a given constit-

uent per fruit. For this reason, growth curves, as shown by the rates of increase in fresh weight of the fruit during the season, have been determined (fig. 1). These curves show almost no growth during the winter months, only slight changes in the pulp weight occurring during December and January, probably because of the rainy season and the comparatively cold weather. Both before and after this period of slow growth, the rate of development was more or less uni-

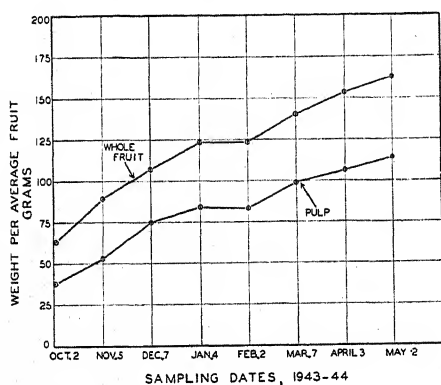


FIG. 1.—Relative weights of whole fruits and pulp of samples of Valencia oranges picked at intervals during season.

form. As would be expected, the increase in weight of the pulp was much greater than that of the peel. Over the 7-month period covered by the samplings, the average weight of the peel, per fruit, increased from 25.3 to 49.2 gm., while that of the pulp increased from 37.7 to 114.5 gm. per fruit (table 1).

The amount of free acid per average fruit was determined on each sample in order to ascertain at what stage of development the organic acids appear in the growing fruit. As shown by the curves in figure 2, the acids increase in the fruit during the early growth period and then remain rather uniform in amount until maturity. The absolute

amount of acid (milligrams per fruit) in the samples of these experiments had nearly reached a maximum by December 7. The slight variations after that date are probably due to experimental and sampling errors. The decrease in concentration of acids per milliliter of juice during ripening was evidently caused by the growth of the fruit and the consequent dilution of the acids present; for as the fruit increases in size, the acids must be distributed through an increasing volume of juice. These results are in agreement with those of BIGELOW and GORE (3), who showed that in Navel oranges the total acid per fruit was produced early in the season and varied only slightly in amount during subsequent growth. Similar results were also noted by McDERMOTT (7).

The total free-acid content per fruit was also determined indirectly by converting the total water content per fruit into its equivalent volume of juice. This can be done if it is assumed that the total water content of the pulp is water of dilution from the juice. In other words, it must be assumed that the acid is uniformly distributed throughout the water content of the pulp. For example, with the April sampling the water content of the pulp averaged 85.04% of the fresh weight of the fruit (106.1 gm.), or 90.2 gm. H_2O per fruit. From the refractive index, the water fraction of the juice was found to be 86.93% and the specific gravity, 1.0528. In 1 ml. of juice there would be $0.8693 \times 1.0528 = 0.915$ gm. H_2O . Therefore, $90.2/0.915$ would represent the milliliters of juice (98.6) per fruit. Since the free acidity (16.45 mg.) per milliliter of juice is known from the potentiometric titration, the free acid (as citric) per fruit can be found:

$$98.6 \times 16.45 = 1621 \text{ mg.}$$

TABLE 1

CHANGES IN SIZE AND IN TOTAL FREE ACIDITY OF VALENCIA ORANGE FRUITS DURING GROWTH

SAMPLING DATE	AV. DIMENSIONS OF FRUITS (CM.)		AV. WEIGHT PER FRUIT (GM.)		DRY MATTER IN PULP (%)	TOTAL FREE ACIDITY (AS CITRIC ACID) PER FRUIT (MG.)	
	Longitudinal axis	Equatorial axis	Peel and pulp	Pulp only		Experimen- tal determi- nation	Calculated
Oct. 2, 1943.....	5.23	4.92	63.0	37.7	14.29	1090	1371
Nov. 5, 1943.....	5.86	5.60	89.0	52.8	13.91	1085	1436
Dec. 7, 1943.....	6.16	5.95	107.2	75.0	13.20	1416	1689
Jan. 4, 1944.....	6.43	6.19	122.8	84.5	13.40	1443	1740
Feb. 2, 1944.....	6.49	6.25	122.2	82.1	13.64	1371	1608
Mar. 7, 1944.....	6.69	6.42	140.1	98.2	14.41	1388	1736
Apr. 3, 1944.....	6.92	6.53	153.0	106.1	14.96	1455	1621
May 2, 1944.....	7.10	6.71	163.7	114.5	15.08	1493	1710

The free acidity value (per fruit) calculated by this method is considerably higher than the actual experimental results. The assumption that the acid is uniformly distributed in the total water of the pulp is undoubtedly the cause of the high results. Not all the moisture in the fruit is extracted as juice, and acid determinations on aliquot portions of the juice produce high results when calculations are made on the basis of the total water content per fruit. The fact that the amount of soluble solids varies considerably in the juice extracted from different portions of the pulp is strong evidence that the acid is not uniformly distributed in the fruit pulp. However (fig. 2), the plotted values for the calculated results of free acidity per fruit give a curve similar to the experimental results.

CITRIC- AND MALIC-ACID CONTENTS OF FRUITS PICKED AT INTERVALS DURING SEASON

The citric- and malic-acid contents of orange juice at different stages of fruit maturity are shown graphically in figure 3. During the period studied (October 2, 1943, to May 2, 1944), the con-

centration of citric acid and citrate decreased from 39.88 to 17.21 mg. per milliliter of juice, but the concentration of malic acid and malate showed only slight variation. However, the malic acid

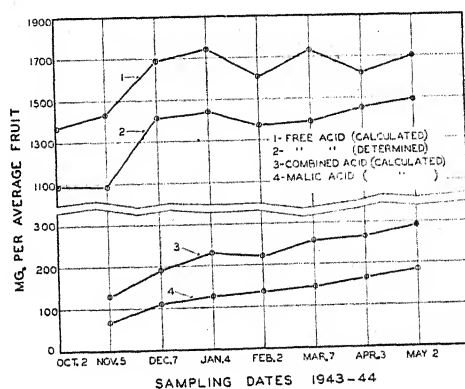


FIG. 2.—Changes in various acid constituents per fruit during fruit development.

showed an increase during the season, if the calculations were made on the basis of the total water content per fruit (fig. 2, curve 4). It must be emphasized that curve 4 was drawn from calculated values and was not experimentally determined. These calculated values have been found to be slightly higher than the experimental results as shown for citric

acid. The important thing, however, is that the total malic-acid content per fruit increased during the growth period, and the concentration of malic acid, in milligrams per milliliter of juice, showed little change.

The free-acid curve (fig. 3), as determined by the potentiometric titration, is lower on the graph but runs parallel with the citric-acid and citrate curve. It follows, therefore, that the decrease in the concentration of the free acid of

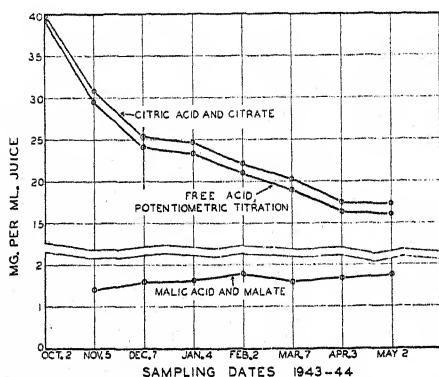


FIG. 3.—Changes in concentrations of various acid constituents in juice during maturation of fruit.

orange juice during ripening is due chiefly to the decrease in citric acid. This result is quite different from that given in figure 2, curve 2, which shows that after the citric acid reached a maximum in the early stage of fruit development, it varied only slightly during the remainder of the ripening period.

RELATION OF FREE ACID TO COMBINED ACIDS IN FRUITS AT DIFFERENT STAGES OF MATURITY

As previously noted, the concentration of free acid decreased with growth and maturity of the fruit. This is shown in table 2, in which the phenolphthalein and potentiometric titration values and the citric-acid determinations (penta-

bromacetone method) decreased with each successive sampling of the fruit. The concentration of combined acids in the juice, however, showed only slight variations over the entire period studied.

The results for the combined acids, as determined by the two very different methods (table 2), are in close agreement. The "calculated" values were determined as the difference between the milliequivalents of free acid and the sum of the milliequivalents of total citric and total malic acids of the samples. The direct determination of combined acids was based on the fact that the salts of organic acids decompose to carbonate salts during ashing. The close agreement between the results of the two methods indicates the validity of the quantitative measurements of total citric and total malic acids. The malic acid and the alkalinity of the ash were not determined on the first sampling of fruit, so there are no combined-acid values for that sample.

Since the concentration of the combined acids did not change appreciably as the fruit grew, it is evident that the absolute quantity per fruit increased gradually, as shown in figure 2. The conversion of the concentration of combined acids from a juice basis (milligrams per milliliter) to a fruit basis (milligrams per fruit) was made according to the method explained for the similar conversion of total free acidity. These calculated results are slightly higher than the actual amount of combined acids per fruit, but they show that the combined acids, unlike the free acid, continued to increase in the fruit during growth and ripening. On that basis, it can be assumed that more inorganic minerals were made available for salt formation, since the cation concentration, and not the acid radical, is the

TABLE 2
CHANGES IN CONCENTRATION OF ACID CONSTITUENTS OF ORANGE JUICE DURING GROWTH OF FRUIT

SAMPLING DATE	SOLUBLE SOLIDS (%)	pH	TOTAL FREE ACID (AS CITRIC)			CITRIC ACID (PEN- TABROMACETONE METHOD)		MALIC ACID		COMBINED ACIDS (AS CITRIC)				ACID RADICAL IN FREE FORM (%)	
			Phenol- phthalein titration (mg./ml.)	Potentiometric titration		mg./ml.	me./ml.	mg./ml.	me./ml.	Calculated*		Determined from alkalinity of ash			
				mg./ml.	me./ml.					mg./ml.	me./ml.				
Oct. 2, 1943.....	9.91	2.72	39.24	39.29	0.614	39.88	0.623	†	†	†	†	†	†	91.8	
Nov. 5, 1943.....	9.91	2.81	30.47	29.58	.462	30.84	.482	1.40	0.021	2.62	0.041	2.75	2.74	0.043	89.8
Dec. 7, 1943.....	10.57	2.88	24.44	24.18	.378	25.39	.397	1.58	.024	2.75	.043	2.68	2.68	.042	89.1
Jan. 4, 1944.....	10.83	2.91	23.69	23.38	.365	24.72	.386	1.62	.024	2.88	.044	3.01	3.01	.047	88.2
Feb. 2, 1944.....	11.37	2.95	21.32	21.07	.329	22.15	.346	1.78	.027	2.81	.044	2.81	2.92	.046	87.1
Mar. 7, 1944.....	12.37	3.03	19.34	19.02	.297	20.28	.317	1.59	.024	2.81	.042	2.89	2.89	.045	86.0
Apr. 3, 1944.....	13.07	3.07	16.66	16.45	.257	17.51	.274	1.68	.025	2.69	.042	2.83	2.83	0.044	85.4
May 2, 1944.....	13.12	3.11	16.35	16.11	0.252	17.21	0.269	1.75	0.026	2.75	0.043				

* Calculated as difference between milliequivalents of free acid and sum of milliequivalents of total citric and malic acids present.

† Data not determined.

limiting factor in the formation of organic salts.

The two curves of figure 4 illustrate the relation of pH to the concentration of free acid and also to the free-acid—combined-acid balance. For curve A, the term "free acid" indicates the percentage of the total-acid radical present in the juice as free acid. In equation form, with acidity values expressed in milliequivalents,

$$\frac{\text{Free acidity}}{\text{Total citric acid} + \text{total malic acid}} \times 100 = \text{percentage of free acid.}$$

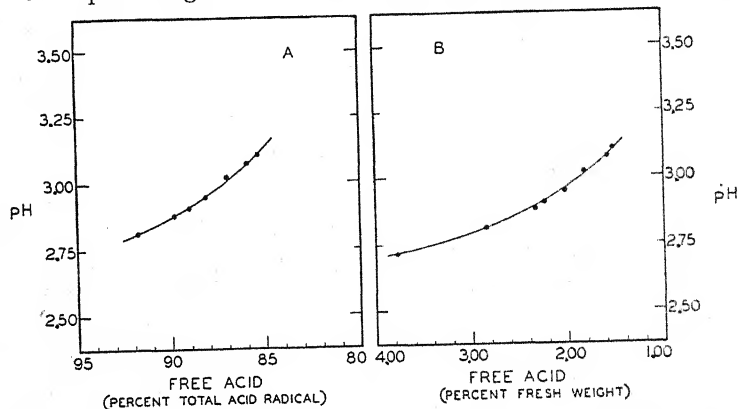


FIG. 4.—Relation of pH to free acid (A) expressed as percentage of total-acid radical of juice, and (B) calculated as percentage of fresh weight. Points on curves represent samples picked between November 5, 1943, and May 2, 1944, for curve A; and between October 2, 1943, and May 2, 1944, for curve B.

As the fruit matured, the percentage of acid in the free state decreased and the pH of the juice increased. The rise in pH was the result of the change in the value $\log(\text{salt/acid})$, since the acid concentration decreased during ripening and the salt (combined-acid) concentration remained nearly constant.

AMERINE and WINKLER (1) have shown that the amount of tartrates present as free acid in grapes was reduced from a range of 50–80% to a range of 10–20% during growth and ripening of the fruit. They attributed the gradual

increase in pH during ripening to changes in the ratio of acid salt to free acid. This change in ratio is due mostly to the change in free acid, for BIOLETTI *et al.* (4) have noted that the rate of increase of cream of tartar during ripening could not account for the decrease in free acidity.

Curve B (fig. 4) shows the relation between pH and the free acid of the juice, calculated as percentage of fresh weight. The values forming this curve were determined on fruit samples picked from trees in the same locality. The variables were thus reduced to a minimum. If the

pH is plotted against the percentage of acid in the juice of fruits grown on different rootstocks and in different locations, the variations are more pronounced. To illustrate with an actual but extreme example (2), juice from Washington Navel oranges grown on Rough-lemon rootstock showed 0.80% total acids (in terms of citric acid), with a pH of 3.52, while juice from Navel oranges grown on Trifoliate orange showed 1.21% total acids, with a pH of 3.46. Although there was a 34% difference in the total acidity of these two juice samples, the pH values

differed only slightly. These two samples of Navel oranges were picked on the same day (March 3, 1942), from two different blocks in the same grove. It is very probable that several variables produce the change in the relation of pH to the percentage of free acid (fresh weight of juice).

The following equation (5) is approximate for calculating the pH of weak acids:

$$\text{pH} = \text{pK}_a + \log \frac{\text{salt}}{\text{acid}},$$

where the term "salt" indicates that of the organic acid present. Since the citric- and malic-acid systems in orange juice can be classed as weak acids, this equation may be applied. Over the concentration changes occurring in the juice, the variation pK_a is very slight; it may therefore be assumed to be a constant in this discussion. That leaves the factor $\log (\text{salt}/\text{acid})$ as the only one that may vary the pH.

The dilution effect of the water in the juice is undoubtedly an important variable. This water content may vary considerably and thus cause variations in the concentration of the acid although the absolute amount of acid present remains constant. The variations in water content do not change the value of $\log (\text{salt}/\text{acid})$, however, as the salt and acid would be diluted or concentrated in the same proportions. The pH of the juice would therefore remain constant, but the concentration of the acid could vary over a wide range.

The opposite may also occur; that is, the pH may vary while the acid concentration remains unchanged. This effect would be caused by the variation in value of the factor $\log (\text{salt}/\text{acid})$. If the

acid concentration remains constant, then the numerator, or salt concentration, must cause the pH variation. It is probable that with fruit from many different trees and groves the amount of salt in the juice would vary enough to produce a significant variation in pH.

With a system such as orange juice, a combination of variables very likely produces the wide variation in the relation of pH to the percentage of acidity on a fresh-weight basis. For example, the extent of dilution could result in a decrease in concentration of the acid, and at the same time a change in the absolute amount of salt might change the pH in the direction of increased acidity. Other factors probably enter into the relation, but the two variables just discussed are considered the most important.

Summary

The maximum amount of free acid in Valencia orange fruits was found to develop early in the season and to change very little from that time on. The concentration of free acids in the juice (milligrams per milliliter), however, lessens considerably during fruit development. This decrease in free acidity, with the corresponding increase in pH, was due chiefly to the decrease in concentration of free citric acid. Although the malic-acid concentration in the juice (milligrams per milliliter) stayed nearly uniform during the season, the actual amount in the fruit increased. The concentration of combined acids remained nearly uniform in the fruit, but the absolute amount per fruit increased. The amounts of combined acids determined from the alkalinity of the ash were in agreement with the values determined from the difference between the total-

and free-acid radicals. During ripening, the changes in pH of the juice were definitely related to changes in percentages of the total-acid radical in the free form. A similar relation was noted between

pH and the percentage of free acid expressed on a fresh-weight basis.

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GROWTH, RUBBER STORAGE, AND SEED PRODUCTION BY GUAYULE AS AFFECTED BY BORON SUPPLY

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Introduction

Crop plants vary with respect to the optimum amount of boron they require for vegetative growth and reproduction and with respect to their ability to tolerate wide variations in their boron supply (2). Symptoms of boron toxicity are exhibited in the vegetative growth of such crops as lettuce and kidney bean when these are grown in sand culture and supplied with a nutrient containing 0.8-1.0 p.p.m. of boron, while others—such as celery, sugar beet, and alfalfa—grow vigorously when supplied with

several times this amount (2). Seed production by a number of crop plants, particularly legumes, is known to be greatly reduced when the plants are grown with an inadequate boron supply, and their vegetative growth is also decreased by boron starvation (7).

Whereas most crop plants which have been studied as to their boron requirement are mesophytic, guayule, indigenous to dry regions, is a xerophytic type of plant. Also, the boron content of the soil and irrigation waters varies widely in the different localities within the United States which are suited to the cultivation of guayule (8). Experiments were undertaken to observe some of the responses that result when this plant is subjected to different levels of boron

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nutrition and to determine its tolerance toward variation in the boron supply.

METHODS.—In a preliminary experiment, 100 uniform transplants (variety 593) of guayule were selected from a large number obtained from a nursery near Salinas, California. They were planted in carefully washed, grade 8 Berkley quartz sand contained in glazed earthenware crocks. The crocks were drained by means of a hole in the bottom, which was covered with loosely packed glass wool to prevent loss of sand. The behavior of guayule plants grown in a boron-free nutrient indicated that no effective amount of boron was derived from either the quartz sand or the crock. The plants were divided into two equal lots. One lot was supplied daily with nutrient made with distilled water and containing 8.75 ml. of 0.5 molecular KH_2PO_4 and MgSO_4 per liter, respectively, and 12.15 ml. of 0.5 mol. $\text{Ca}(\text{NO}_3)_2$ per liter. Iron, manganese, copper, and zinc were also supplied to the plants in concentrations of 0.5 p.p.m. of nutrient solution. The remaining plants were supplied with the same solution but containing sufficient boric acid to make a concentration of 0.5 p.p.m. of boron. After growing for 6 weeks under greenhouse conditions, the boron-deficient plants were divided into five groups, and during the following 8 weeks they were given the same basic nutrient but containing 0.0, 0.25, 0.5, 1.0, and 4.0 p.p.m. boron, respectively.

In a subsequent experiment, seeds were germinated, in September, 1942, in a thin layer of sand which was spread over soil. When approximately 2 inches tall, selected plants were dug and the soil carefully washed from their roots. The seedlings were then planted individually in 2-gallon glazed earthenware crocks containing a mixture of coarse

sand and fine gravel which had been washed carefully four times previous to use in order to eliminate soil particles. The crocks were rounded on the lower side and drained by means of a hole approximately 1 inch in diameter located in the center of the bottom. A screen covered with asphalt paint was placed over the hole to prevent loss of sand gravel. The crocks were arranged in a greenhouse in such a way that seven treatments could be applied as randomized blocks. A basic nutrient was made up of 17.5 milliequivalents of $\text{Ca}(\text{NO}_3)_2$, 2.6 me. of KH_2PO_4 , 2.6 me. of MgSO_4 , and 0.3, 0.25, 1.0, and 1.0 mg. of iron, manganese, copper, and zinc, respectively, per liter of distilled water. Seven boron levels were prepared by adding to aliquots of the basic nutrient sufficient boric acid to make solutions containing 0.0, 0.01, 0.1, 0.5, 2.0, 5.0, and 10.0 p.p.m. of boron. These solutions were applied daily in amounts sufficient to flush the cultures. Distilled water was added at intervals during the day to maintain optimum moisture conditions.

The plants were grown under greenhouse conditions until April, 1943, when they were moved outdoors. At this time it was necessary, in making up the nutrient solution, to substitute tap water which contained 0.004 p.p.m. of boron, thus increasing the amount of boron at each nutrient level by this amount. The plants were grown outdoors until October, when they were again returned to a greenhouse and grown with the same nutrient treatments, using tap water and under a controlled day temperature of 55°–65° and a night temperature of 35°–45° F.

Seeds were collected at approximately weekly intervals during the summer (June 1 to September 1) and fall (September 1 to October 16) of 1943. These

were cleaned, then sorted into three sizes by separating those that failed to pass an 8-mesh per inch screen, those that passed the 8-mesh but failed to pass a 10-mesh, and those that passed a 10-mesh screen. The seeds were stored at room temperature for several months in sealed paper envelopes until they were tested for germination as previously described (6). In March, 1944, the plants were harvested and their fresh weights determined. The leaves were removed, and the stems and roots were cut into small pieces and dried for 24 hours in a well-ventilated oven at 60°C. Rubber and resin determinations of stems and roots were made by the SPENCE and CALDWELL method (10). Chemical determinations of the amount of boron in the stems and roots and in the seeds were made by the WILCOX (14) and the BERGER and TRUOG (1) methods.

At the time of final harvest, eight plants from each of four different levels of boron supply (0.004, 0.1, 0.5, and 10.0 p.p.m.) were sampled for the purpose of anatomical study. A segment approximately 1 cm. in length was secured from the base of the main stem of each plant, all pieces being taken from the lowermost 3 cm. of the stem in order that the sections should be comparable. The lower limit of this region was identified in part by the crowded leaf scars just above the cotyledonary node but mainly by the appearance of medullary resin canals, which are present in the stem but not in the hypocotyl (4). The manner of preparing slides and determining tissue areas has been described earlier (13). Data were obtained for the cross-sectional area of the wood, consisting of the pith and xylem, and of the bark, consisting of those tissues external to the xylem but internal to the cork. Within the bark, the major rubber-

storing portion of the stem, fiber tissue decreases the potential storage area. Measurements of the area occupied by fiber were therefore made.

In an experiment designed to illustrate the toxic effects of relatively high concentrations of boron, the same cultural methods were used as previously described. Field-grown seedlings were transplanted to a sand-gravel mixture and supplied with nutrient containing 8.75 ml. of 0.5 mol. KH_2PO_4 and MgSO_4

TABLE 1
LEAF GROWTH AND BORON CONTENT OF STEMS AND LEAVES OF GUAYULE SUPPLIED NUTRIENT CONTAINING DIFFERENT LEVELS OF BORON

BORON IN NUTRIENT SOLUTION (P.P.M.)		DEAD LEAVES (%)	BORON IN DRY MATTER (P.P.M.)	
First 3 weeks	Following 4 weeks		Stems	Green leaves
0.0.....	0.0	22	22
0.5.....	0.5	8.0	25	78
10.0.....	10.0	15.4	62	517
2.0.....	20.0	23.2	82	817
5.0.....	40.0	37.5	171	1189

per liter, respectively, and 12.15 ml. of 0.5 mol. $\text{Ca}(\text{NO}_3)_2$ per liter. The basic nutrient solution also contained 0.5 p.p.m. of zinc, iron, copper, and manganese, respectively. Boric acid was added to the nutrient to obtain solutions with boron concentrations of 0.0, 0.5, 2.0, 5.0, and 10.0 p.p.m. These were applied respectively to five groups, each consisting of eight selected plants grown in a greenhouse. Since no toxicity symptoms were apparent, after 3 weeks of treatment the amounts of boron were increased in the case of the plants of two groups, as indicated in table 1. At the end of the following 4-week period all plants were harvested, and the weight of dead leaves per plant and also the boron content of green leaves and of the stems and roots were determined.

Results

RESPONSE TO LACK OF BORON.—In the preliminary experiment designed to show responses to the lack of boron, the guayule transplants exhibited characteristic boron-deficiency symptoms after being supplied with a boron-free nutrient for 2 weeks after transplanting. The leaves of the plants failed to grow and became curled and deformed. During the third and fourth weeks of treatment, pinkish brown spots appeared at the margins of the older leaves, followed by necrotic areas, a series of symptoms observed to

4.2 gm., and they produced an average of one flower head per plant. Those supplied with nutrient containing less than 1.0 p.p.m. made no appreciable top growth and failed to produce an appreciable number of flower heads. Those supplied with 1.0 p.p.m. made a limited amount of growth (average fresh weight 13.7 gm.) but failed to produce many flowers. Those supplied with a solution containing 4 p.p.m. recovered to a greater extent (average fresh weight 23.9 gm., fig. 1) and produced several flower stalks per plant. The average weight of plants

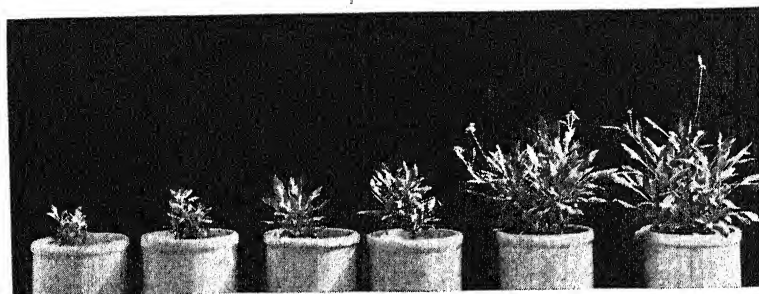


FIG. 1.—Recovery of guayule from boron deficiency. Two plants on left grown for 14 weeks with nutrient lacking boron. Center plants grown for 6 weeks without boron, then supplied with nutrient containing 1 p.p.m. for following 8 weeks. Plants on right grown without boron for 6 weeks, then supplied with nutrient containing 4 p.p.m. for following 8 weeks.

be characteristic of severe boron deficiency in guayule. A few of the uppermost leaves expanded to some extent, but these were chlorotic and failed to attain full size as compared with similar leaves of plants grown with an adequate boron supply. In the absence of boron, the plants made no appreciable top growth and failed to flower during the first 6 weeks of treatment, while others that received boron flowered during this period. Some of the plants lacking boron were then given daily applications of nutrient containing different levels of boron in order to test their ability to recover from the deficiency. During the following 8 weeks, the average weight of plants grown without boron was only

supplied with boron and a complete nutrient throughout the experiment was 61.6 gm., and they produced an average of 89.8 flower heads per plant.

RESPONSE TO DIFFERENT LEVELS OF BORON.—The growth of seedlings was greatly retarded in the case of all plants given boron-free nutrient during the first 6 months of the experiment. These boron-deficient plants were then supplied with nutrient which contained 0.004 p.p.m. of boron, and they made some growth; but in the following 10 months they were smaller and much more bushy than were the plants which received greater amounts (fig. 2). Their leaves were relatively small, and green instead of silvery green, as were those of

plants which received greater amounts of boron. Increasing the amount in the nutrient from 0.004 to 0.1 p.p.m. was associated with a significant increase in

receiving nutrient containing 0.1–2.0 p.p.m. (table 2). Plants suffering from toxicity exhibited numerous dead leaves on the lower branches at intervals during

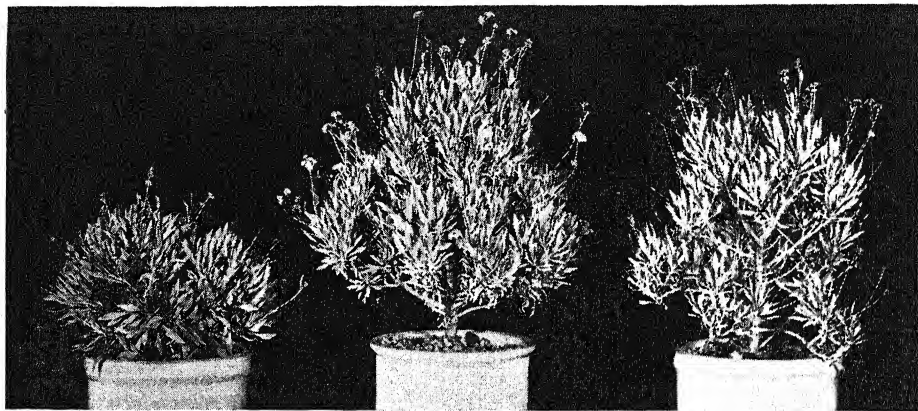


FIG. 2.—Guayule plants grown in gravel culture and supplied different amounts of boron. Left, supplied nutrient solution lacking boron for 6 months, then supplied solution containing 0.004 p.p.m. boron for following 10 months. Center, nutrient containing 0.5 p.p.m. for 16 months. Right, 10 p.p.m. of boron for 16 months.

TABLE 2

EFFECT OF NUTRIENT CONTAINING DIFFERENT LEVELS OF BORON ON PLANT WEIGHT AND ON AMOUNT OF RUBBER AND RESIN PRODUCED BY GUAYULE GROWN IN SAND CULTURE FOR 16 MONTHS

Boron in nutrient (p.p.m.)	Fresh weight per plant (gm.)	Dry weight, stems and roots (gm.)	Rubber (%)	Rubber per plant (gm.)	Resin (%)	Boron in roots and stems (p.p.m.)
0.004.....	203	55	3.32	1.8	3.84	18
0.014.....	255	69	3.86	2.7	4.07	18
0.1*.....	341	100	4.66	4.7	4.64	19
0.5.....	367	106	4.41	4.7	4.33	23
2.0.....	343	102	4.43	4.5	4.63	31
5.0.....	292	91	4.02	3.7	4.66	47
10.0.....	274	80	3.64	2.9	4.10	48

* For convenience, the amount of boron in tap water is not included in expressing concentrations greater than 0.014 p.p.m.

growth. Concentrations of boron ranging between 0.1 and 2.0 p.p.m. had no significant effect on the vegetative growth of the plants—on the basis of dry weight. The growth of plants supplied with nutrient containing more than 2.0 p.p.m. was significantly less than that of plants

the experiment; and they developed an open type of branching (apparently due to inhibition of lateral bud growth), in contrast to dense branching associated with boron deficiency (apparently due to inhibition of terminal but not lateral buds).

The boron content of roots and stems was relatively low as contrasted with that of seeds, particularly the seeds of plants grown with nutrient containing an ample amount (tables 1, 3). In the preliminary experiment, in which field transplants were grown in gravel culture, the leaves were also found to contain a relatively large amount of boron as contrasted with the stems, provided the plants were supplied with a nutrient

ber in the stems and roots and also the total rubber output of the plant (table 2). Variations in boron supply had only a very slight effect on the percentage of resin in the stems and roots.

STEM ANATOMY.—Both a deficiency and an excess of boron in the nutrient solution significantly decreased growth of the stems in diameter (table 4). Increasing the boron content of the nutrient from 0.1 to 10 p.p.m. did not sig-

TABLE 3

EFFECT OF BORON SUPPLY ON AVERAGE NUMBER, SIZE, AND BORON CONTENT OF SEEDS PRODUCED DURING DIFFERENT SEASONS OF THE YEAR BY GUAYULE PLANTS GROWN IN GRAVEL CULTURE. FIGURES REPRESENT AVERAGE NUMBER OF SEEDS PRODUCED PER PLANT DURING SUMMER (JUNE 1 TO SEPTEMBER 1) AND FALL (SEPTEMBER 1 TO OCTOBER 16), AND PERCENTAGE OF LARGE, MEDIUM, AND SMALL SEEDS

BORON IN NUTRIENT (P.P.M.)	NO. OF SEEDS PRODUCED		PERCENTAGE						BORON CONTENT SUMMER COLLEC- TION (P.P.M.)
			Small		Medium		Large		
	Summer	Fall	Summer	Fall	Summer	Fall	Summer	Fall	
	0.004.....	1380	626	78.5	42.0	20.9	52.8	0.7	
0.014.....	2350	892	49.8	39.2	48.6	56.5	1.6	4.2	27
0.1.....	2519	1209	18.6	13.5	64.6	70.5	16.9	16.0	30
0.5.....	2220	1183	20.1	14.2	62.4	68.1	17.5	17.7	43
2.0.....	2282	1109	15.5	14.5	59.3	66.9	25.2	18.6	71
5.0.....	2204	1062	17.7	16.5	53.7	65.6	28.6	18.0	82
10.0.....	2138	881	15.6	13.3	56.0	69.8	28.5	16.9	212

containing an ample supply of boron. An increase in the amount supplied to the plants was accompanied by a corresponding increase in boron concentration in the stems, and particularly in the leaves. Slight toxicity symptoms were evident within a period of 7 weeks in plants supplied with a nutrient containing 10 p.p.m., as indicated by the presence of dead leaves, and these symptoms became more pronounced with increasing amounts of boron.

RUBBER AND RESIN.—The application of nutrient containing less than 0.1 or more than 2.0 p.p.m. of boron significantly reduced the concentration of rub-

nificantly change the percentage of bark or wood in the cross-sectional areas. The percentage area of bark in cross-sections of stems of plants supplied with nutrient containing 0.004 p.p.m. was significantly greater than that of others supplied with larger amounts of boron. A relatively large proportion of fiber was observed in the bark, both in those plants that showed boron deficiency and in those that showed toxicity symptoms.

The cellular structure of the lower stem showed none of the irregularities in cell growth or differentiation, or the disorganization and necrosis described by others (11, 12) for plants grown under

more severe stress of boron deficiency or toxicity. In plants deficient in boron (supplied 0.004 p.p.m. in the nutrient) compared with those receiving nutrient containing 0.1 p.p.m., parenchyma tissue of the bark appeared to be similar in cell size and tissue organization. The area of undifferentiated tissue adjacent to the cambium, as measured from the innermost mature phloem fiber to the outermost mature xylem, formed a slightly greater proportion of the stem tissue in plants supplied with nutrient

of a relatively large proportion of medium and large-sized seeds.

The percentage germination of large seeds was significantly greater than that of medium or small-sized seeds (table 5), and that of seeds produced through the use of solutions containing 0.1 p.p.m. boron was significantly greater than that of seeds produced by plants receiving smaller amounts. There was no significant difference in the percentage germination of seeds obtained through the use of nutrient solutions that contained

TABLE 4

EFFECT OF BORON SUPPLY ON CROSS-SECTIONAL AREAS OF STEM AND COMPONENT TISSUES. FIGURES REPRESENT AVERAGES OF EIGHT PLANTS EACH

BORON IN NUTRIENT (P.P.M.)	TOTAL STEM AREA (MM. ²)	WOOD		BARK		BARK FIBERS	
		Area (mm. ²)	Total stem area (%)	Area (mm. ²)	Total stem area (%)	Area (mm. ²)	Bark area (%)
0.004...	133.2	51.87	38.8	82.04	61.3	22.99	28.5
0.1.....	211.6	90.74	42.9	120.77	57.1	29.06	24.3
0.5.....	231.6	98.24	42.5	133.12	57.5	32.34	24.6
10.0.....	148.2	63.76	43.2	84.55	56.9	23.82	28.7

containing 0.004 p.p.m. of boron than it did in stems of those supplied 0.1 p.p.m.

SEEDS.—Plants of a different variety (406) produced a relatively large number of seeds when supplied with nutrient containing a wide range of boron levels—from 0.004 to 5 p.p.m. (table 3). The use of nutrient containing a higher level (10 p.p.m.) did not decrease seed production appreciably, but the application of deficient amounts (0.004 p.p.m.) greatly reduced seed production. Application of nutrient deficient in boron was associated with the production of a relatively high percentage of small seeds, while the use of nutrient containing 0.1–10 p.p.m. resulted in the production

0.1–10.0 p.p.m. The percentage germination of seeds collected during September and October was significantly greater than that of seeds collected during the summer months.

Discussion

In nutrient cultures, the boron requirements of guayule were similar to those of some plants reported by others—such as tobacco, lettuce, soybean, cotton, broad bean, and sunflower (3, 5, 9)—in that it required approximately the same optimal concentration. Guayule apparently differs from most of these plants in that it has a slightly wider tolerance range, from 0.1 to approximately 2.0 p.p.m. MCHARGUE (5)

found the optimal range for lettuce to be 0.4–0.8 p.p.m., but 0.9 produced symptoms of toxicity. EATON (3) found that 0.5 was optimal for the sunflower and that symptoms of toxicity appeared in plants grown with a 1.0 p.p.m. boron

outside the limits of its tolerance range (0.1–2.0 p.p.m.), the vegetative growth of gravel-culture guayule plants was reduced. Since this decrease in the dry weight of the plants was associated with decrease in the rubber content of the

TABLE 5

EFFECT OF BORON SUPPLY ON GERMINATION OF SEEDS FROM PLANTS GROWN IN GRAVEL CULTURES COLLECTED DURING SUMMER (JUNE 1 TO SEPTEMBER 1) AND FALL (SEPTEMBER 1 TO OCTOBER 16)

COLLECTION DATE	BORON IN NUTRIENT SOLUTION (P.P.M.)						
	0.004	0.014	0.1	0.5	2.0	5.0	10.0
Large-sized seeds							
Summer.....	30.75	18.75	49.25	58.50	49.50	41.75	45.75
Fall.....	34.75	35.25	57.50	65.50	61.75	59.25	59.75
Medium-sized seeds							
Summer.....	8.50	12.75	26.00	29.75	34.25	30.50	23.25
Fall.....	32.75	32.00	39.75	49.25	46.25	41.75	42.50
Small-sized seeds							
Summer.....	1.00	2.25	4.50	5.75	5.75	4.75	7.25
Fall.....	10.00	9.75	16.25	14.75	17.00	18.50	18.25
Mean of all sizes							
Summer.....	13.50	11.25	26.50	31.25	29.75	25.75	25.50
Fall.....	25.75	25.75	37.75	42.50	41.75	39.75	40.25

supply. EATON also stated that 0.5 p.p.m. is sufficient for most plants, or may be excessive for some. The relatively wide boron-tolerance range of guayule may enable it to grow in some of the high-boron soils found within the cultivated guayule range, or when irrigated with water which has a relatively high boron content.

When supplied with amounts of boron

roots and stems, the effect on total rubber output was marked. Plants supplied with nutrient containing a deficient amount of boron (0.004 p.p.m.) produced 60% less, and those supplied with nutrient containing an excessive amount (10.0 p.p.m.) produced 35% less, rubber than did others given an optimum supply.

The growth resulting from cambial

activity, as seen in the total stem area, is also unfavorably affected by either a deficiency or an excess of boron. With excess (10 p.p.m.) there was an over-all decrease in the formation of both wood and bark tissues. In the case of deficiency (0.004 p.p.m.), the significantly greater proportion of bark to wood (both composed mainly of secondary tissues) suggests that cambial activity was altered as the result of treatment. Histological symptoms, characteristic of pronounced boron toxicity or deficiency, were absent from the meristematic tissues of the lower stems.

It is reported (7) that the quantity of seeds produced by legumes was greatly reduced when the plants were grown with an inadequate boron supply but that their quality with respect to germination was not changed. Boron deficiency (0.004 p.p.m.) in guayule was associated with the production of relatively few seeds and also with a decrease in their quality, reflected in both size and percentage of germination. Relatively high concentrations of boron occurred in the seeds, as compared with the stems, and the amount of boron in the seeds varied widely, depending upon the amount available to the plant. Seeds containing less than 30 p.p.m. of boron were relatively low in percentage germination. Seeds that contained 30 p.p.m., however, were approximately the same with respect to germination as other seeds that contained 212 p.p.m. These results indicate that the quality of seeds as to size and germination produced by guayule grown on soils having a relatively high boron content would depend largely upon factors other than the available boron supply.

Summary

1. Plants of guayule were grown from early seedling stage in gravel culture for

a period of 16 months, during which time they were supplied with nutrient solutions containing 0.0-10.0 p.p.m. of boron. In other experiments, somewhat older seedlings were transplanted from the field and grown in sand or gravel cultures to determine the effects of deficient or excessive amounts of boron (0.0-40.0 p.p.m.) when these were supplied by means of nutrient solutions.

2. The plants showed marked boron-deficiency symptoms when supplied with a boron-free nutrient, but they subsequently grew vigorously and produced seeds when supplied with a nutrient solution containing 4 p.p.m.

3. According to results based on vegetative growth in gravel cultures, guayule has a tolerance range of from 0.1 to 2.0 p.p.m. of nutrient solution, a range somewhat wider than that reported for most crop plants of a mesophytic type.

4. Data on percentage of rubber in gravel-culture plants show an optimum boron requirement of 0.1-2.0 p.p.m., a concentration range similar to that required for maximum vegetative growth and seed production. The concentration of rubber in the stems and roots of boron-deficient plants was lower than that of similar parts of plants grown with an adequate supply.

5. Since boron deficiency was associated with both reduced vegetative growth and lower percentage of rubber, the total rubber output of boron-deficient plants was relatively small. An excessive amount of boron (10 p.p.m. in the nutrient) was also associated with a decrease in rubber output. Over the range studied, the amount of boron supplied to the plants had only a very slight effect on the concentration of resins in their stems and roots.

6. The stem diameters of gravel-culture plants showed that cambial activity was somewhat reduced in the plants

receiving nutrient containing 10.0 p.p.m. of boron, and also in the stems of plants that received a deficient amount. The proportions of pith, xylem, bark, and bark fiber in the basal region of the stem were not altered as the result of supplying the plants with nutrient containing various levels of boron (0.01–10.0 p.p.m.). In stems of plants grown with a limited supply (0.004 p.p.m.) the area of bark was greater, in proportion to the amount of wood produced, than in comparable segments of stem from plants grown with an adequate boron supply.

7. Boron-deficient plants produced fewer seeds than did plants supplied with an adequate amount. Seeds produced by boron-deficient plants were smaller and their germination was lower

than those from plants grown with an adequate supply. With respect to size, number, and percentage germination, the quality of seeds produced by plants supplied with nutrient containing an excessive amount of boron (10 p.p.m.) was equal to that produced by plants grown with a moderate supply (0.1 p.p.m.). Boron accumulated in the seeds (and also leaves) of the plants grown with an adequate boron supply.

All rubber and resin determinations were made by R. L. HOLMES, boron determinations by GLEN EDGINGTON, and statistical analyses by MARY W. SHANOR.

BUREAU OF PLANT INDUSTRY, SOILS
AND AGRICULTURAL ENGINEERING
BELTSVILLE, MARYLAND

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THE KNOWN GEOGRAPHIC DISTRIBUTION OF THE MEMBERS OF THE VERBENACEAE AND AVICENNIACEAE. SUPPLEMENT 2

HAROLD N. MOLDENKE

Since the publication of my original compilation of the known geographic distribution of the 2966 accepted members of the families Verbenaceae and Avicenniaceae¹ and its first supplement,² based chiefly on an examination of 75,000 herbarium specimens in 142 private and institutional herbaria, continued collecting by numerous colleagues and correspondents and the examination of more herbarium material have brought to light 312 additional records which are presented herewith. These represent 33 new country or island records, 73 state or province records, and 206 county or parish records. Four new taxonomic entities are proposed and described.

CANADA:

Ontario

Verbena hastata L. (Algoma and York Counties)

Verbena urticifolia L. (Simcoe County)

Manitoba

Verbena bracteata Lag. & Rodr.

UNITED STATES OF AMERICA:

Maine

Verbena hastata L. (Waldo County)

New York

Verbena hastata L. (Cortland and Rensselaer Counties)

Verbena urticifolia L. (Cortland and Rensselaer Counties)

¹ MOLDENKE, H. N., The known geographic distribution of the members of the Verbenaceae and Avicenniaceae. Pp. 104. New York City. 1942.

² ———, The known geographic distribution of the members of the Verbenaceae and Avicenniaceae. Supplement 1. Pp. 4. New York Botanical Garden. 1943.

Pennsylvania

Verbena hastata L. (Bradford County)

Verbena simplex Lehm. (Centre County)

Virginia

Verbena simplex Lehm. (Henrico County)

Verbena urticifolia L. (Henrico County)

Verbena urticifolia var. *leiocarpa* Perry & Fernald (Campbell County)

West Virginia

Verbena urticifolia L. (Jackson County)

Verbena urticifolia var. *leiocarpa* Perry & Fernald (Wyoming County)

North Carolina

Verbena bonariensis L.

Verbena urticifolia var. *leiocarpa* Perry & Fernald (Catawba County)

South Carolina

Verbena tenuisecta Briq. (Lexington County)

Georgia

Callicarpa americana L. (Oglethorpe County)

Verbena simplex Lehm. (Fulton County)

Verbena tenuisecta Briq. (Ware County)

Florida

Clerodendrum bungei Steud. (Leon County)

Lantana involucrata L. (Palm Beach County)

- Lantana montevidensis* (Spreng.)
Briq. (Citrus County)
Phyla nodiflora (L.) Greene (Collier
and Pinellas Counties)
Verbena maritima Small (Broward
County)
Verbena tenuisecta Briq. (Bay Coun-
ty)

Alabama

- Callicarpa americana* L. (Elmore
County)
Clerodendrum bungei Steud. (Mont-
gomery and Tuscaloosa Counties)
Phyla lanceolata (Michx.) Greene
(Montgomery County)
Verbena tenuisecta Briq. (Butler
County)
Verbena xutha Lehm. (Montgomery
County)

Mississippi

- Verbena rigida* Spreng. (Warren
County)
Verbena xutha Lehm. (Harrison,
Hinds, and Warren Counties)

Ohio

- Verbena simplex* Lehm. (Adams and
Highland Counties)
Verbena urticifolia L. (Ross County)

Illinois

- Phyla lanceolata* (Michx.) Greene
(Alexander, Franklin, Johnson,
McHenry, and Vermilion Coun-
ties)
Verbena bracteata Lag. & Rodr.
(Champaign and Jackson Coun-
ties)
Verbena canadensis (L.) Britton
(Union County)
× *Verbena engelmannii* Moldenke
(Adams County)
Verbena hastata L. (Vermilion Coun-
ty)
× *Verbena rydbergii* Moldenke
(Champaign County)

- Verbena stricta* Vent. (Union County)
Verbena urticifolia L. (Adams, Ver-
milion, and Woodfred Counties)
Verbena urticifolia var. *leiocarpa*
Perry & Fernald (Alexander
County—not "Miami County"
as erroneously stated in Supple-
ment 1)

Indiana

- Phyla lanceolata* (Michx.) Greene
(Bartholomew, Floyd, Hunting-
ton, Jennings, Johnson, Madison,
Miami, Parke, Shelby, Tipton,
and Wabash Counties)
Verbena bracteata Lag. & Rodr. (Cass,
Grant, Howard, Miami, Mont-
gomery, and Starke Counties)
× *Verbena engelmannii* Moldenke
(Boone, Carroll, Cass, Franklin,
and Hancock Counties)
Verbena hastata L. (Bartholomew,
Boone, Clinton, Dearborn, Frank-
lin, Fulton, Gibson, Hancock,
Howard, Lawrence, Madison,
Miami, Randolph, Sullivan, Vigo,
and Wabash Counties)
Verbena simplex Lehm. (Carroll, Kos-
ciusko, and Lawrence Counties)
Verbena stricta Vent. (Howard,
Miami, and Tipton Counties)
Verbena urticifolia L. (Boone, Clin-
ton, Crawford, Franklin, Hamil-
ton, Hendricks, Kosciusko, Madi-
son, Miami, Montgomery, Ohio,
Owen, Ripley, Tippecanoe, and
Wabash Counties)
Verbena urticifolia var. *leiocarpa*
Perry & Fernald (Brown, Decatur,
Dearborn, Greene, Hamilton, Han-
cock, Jefferson, Miami, Monroe,
Parke, and Randolph Counties)

Kentucky

- Verbena hastata* L. (Hopkins County)
Verbena simplex Lehm. (Larue
County)

Tennessee

Verbena canadensis (L.) Britton
(Wilson County)

Verbena simplex Lehm. (Wilson
County)

Michigan

Verbena bracteata Lag. & Rodr.
(Menominee County)

Verbena hastata L. (Mackinac and
Menominee Counties)

Verbena stricta Vent. (Menominee
County)

Verbena urticifolia L. (Menominee
County)

Wisconsin

Verbena bracteata Lag. & Rodr.
(Walworth County)

Verbena hastata L. (Vilas County)

Verbena urticifolia L. (Lafayette
County)

Minnesota

Verbena bracteata Lag. & Rodr.
(Rice County)

Verbena hastata L. (Rice County)

Verbena stricta Vent. (Fillmore
County)

Verbena urticifolia var. *leiocarpa*
Perry & Fernald (Rice County)

South Dakota

Verbena bracteata Lag. & Rodr.
(Custer and Pennington Coun-
ties)

Verbena hastata L. (Pennington
County)

Missouri

× *Verbena illicita* Moldenke (Coop-
er County)

Arkansas

Verbena bracteata Lag. & Rodr.
(Garland County)

Verbena canadensis (L.) Britton
(Chicot, Conway, Franklin, Pope,
and Yell Counties)

Verbena halei Small (Calhoun Coun-
ty)

× *Verbena illicita* Moldenke (Baxter
County)

Verbena litoralis H.B.K. (Bradley
County)

× *Verbena moechina* Moldenke
(Newton County)

Verbena stricta Vent. (Baxter, John-
son, Pope, Searcy, and Van Buren
Counties)

Verbena tenuisecta Briq. (Calhoun
County)

Verbena urticifolia L. (Cross, Gar-
land, Johnson, Marion, Pope,
and Yell Counties)

Verbena urticifolia var. *leiocarpa*
Perry & Fernald (Newton and
Phillips Counties)

Louisiana

Callicarpa americana L. (Livingston
Parish)

Phyla incisa Small (Bossier Parish)

Verbena bonariensis L. (Tangipahoa
and Terrebonne Parishes)

Verbena halei Small (Iberville, Saint
James, and Tangipahoa Parishes)

Verbena scabra Vahl (Tangipahoa
Parish)

Verbena urticifolia L. (Bossier Parish)

Verbena xutha Lehm. (Saint James
Parish)

Montana

Verbena bracteata Lag. & Rodr.
(Park County)

Wyoming

Verbena stricta Vent. (Weston Coun-
ty)

Utah

Phyla cuneifolia (Torr.) Greene
(Emery County)

Nevada

Verbena bracteata Lag. & Rodr. (Elko
County)

Nebraska

- Phyla cuneifolia* (Torr.) Greene
(Hall County)
Phyla lanceolata (Michx.) Greene
(Cass and Washington Counties)
Verbena bipinnatifida Nutt. (Furnas
County)
Verbena bracteata Lag. & Rodr.
(Washington County)
Verbena canadensis (L.) Britton
(Furnas County)
Verbena hastata L. (Washington
County)
Verbena stricta Vent. (Adams, Cher-
ry, Nemaha, and Washington
Counties)
Verbena urticifolia var. *leiocarpa*
Perry & Fernald (Washington
County)

Oklahoma

- Verbena bracteata* Lag. & Rodr.
(Bryan County)
Verbena canadensis (L.) Britton
(Bryan County)
Verbena stricta Vent. (Bryan County)
Verbena urticifolia L. (Bryan Coun-
ty)

Texas

- Lantana horrida* H.B.K. (Llano
County)
Phyla incisa Small (Edwards and
Presidio Counties)
Tetraclea coulteri A. Gray (Presidio
County)
Verbena bipinnatifida Nutt. (Gon-
zales County)
Verbena canadensis (L.) Britton
(Culberson County)
Verbena halei Small (Grimes Coun-
ty)
Verbena neomexicana var. *xylopoda*
Perry (Presidio County)

Arizona

- Verbena ciliata* Benth. (Mohave
County)

California

- Phyla incisa* Small (Kings County)
Phyla lanceolata (Michx.) Greene
(Kings County)
Verbena gooddingii var. *nepetifolia*
Tidestr. (San Bernardino County)

MEXICO:

- Phyla nodiflora* (L.) Greene (Micho-
acán)
Phyla scaberrima (A. L. Juss.) Mol-
denke (Chiapas)
Priva aspera H.B.K. (Tamaulipas)
Priva mexicana (L.) Pers. (Tamau-
lipas and Zacatecas)
Stachytarpheta mutabilis var. *viola-*
cea Moldenke (Michoacán)
Stachytarpheta violacea Miranda
(Puebla)*
Tetraclea coulteri A. Gray (Tamau-
lipas)
Verbena ehrenbergiana Schau. (Ta-
maulipas)
Verbena elegans var. *asperata* Perry
(Coahuila and Zacatecas)
Verbena gracilis Desf. (Coahuila)
Verbena litoralis H.B.K. (Tamauli-
pas)
Verbena perennis Wooton (Tamau-
lipas)

COSTA RICA:

The "+" should be deleted after the
name *Citharexylum macradenium*
Greenm., since this species, for-
merly known only from Costa
Rica, has now been found in
Panama.

PANAMA:

Citharexylum macradenium Greenm.
(Coclé)
The "+" should be deleted after the
name *Citharexylum macrochlamys*
Pittier, since this species, formerly
known only from Panama, has
now been found in Colombia.

ST. BARTHOLOMEW:

- Lantana camara* var. *flava* (Medic.)
Moldenke
Lantana camara var. *sanguinea*
(Medic.) L. H. Bailey
Phyla nodiflora (L.) Greene
Phyla nodiflora var. *reptans*
(H.B.K.) Moldenke

COLOMBIA:

- Aegiphila aculeifera* Moldenke (Mé-
ta)
Aegiphila martinicensis Jacq. (San-
tander Norte)
Avicennia nitida Jacq. (Nariño)
Citharexylum macrochlamys Pittier
(El Cauca)
Duranta repens L. (Nariño)
Duranta sprucei Briq. (Putumayo)
Lantana armata Schau. (Santander
Norte)
Lantana boyacana Moldenke (San-
tander Norte)
Lantana canescens H.B.K. (San-
tander Norte)
Lantana canescens var. *integrifolia*
Moldenke (Cundinamarca)*
Lantana glandulosissima Hayek
(Santander Sur)
Lantana moritziana Otto & Dietr.
(Putumayo and Santander Norte)
Lantana rugulosa H.B.K. (Putu-
mayo)
Lippia schlimii Turcz. (Caldas and
Santander Sur)
Phyla scaberrima (A. L. Juss.)
Moldenke (El Valle)
Stachytarpheta cayennensis (L. C.
Rich.) Vahl (Santander Norte)
Stachytarpheta cayennensis f. *al-
biflora* Moldenke (El Cauca)
Verbena litoralis H.B.K. (Santander
Norte)

VENEZUELA:

- Bouchea prismatica* (L.) Kuntze
(Carabobo)

- Citharexylum spinosum* L. (Aragua)
Lantana camara var. *mista* (L.) L.
H. Bailey (Bolívar)
Lantana trifolia L. (Carabobo)
Lippia berterii Spreng. (Bolívar)
Phyla betulaefolia (H.B.K.) Greene
(Carabobo)
Stachytarpheta australis Moldenke
(Carabobo)
Stachytarpheta cayennensis f. *albi-
flora* Moldenke (Bolívar)
Stachytarpheta mutabilis (Jacq.)
Vahl (Lará)
Vitex orinocensis var. *multiflora*
(Miq.) Huber (Carabobo)

ECUADOR:

- Duranta repens* L. (Manabí)
Lantana scabiosaeiflora H.B.K.
(Azuay)
Verbena microphylla H.B.K. (Leon)

PERU:

- Castelia cuneato-ovata* Cav. (Tacna)
Duranta sprucei Briq. (Puño)
Junellia juniperina var. *grisea* (I.
M. Johnst.) Moldenke (Tacna)
Verbena berterii (Meisn.) Schau.
(Ayacucho and Huancavelica)
Verbena clavata Ruiz & Pav. (Tacna)
Verbena glabrata H.B.K. (Ayacucho)
Verbena variabilis Moldenke (Hu-
ancavelica)*

BRAZIL:

- Aegiphila scandens* Moldenke (Ama-
zonas)

BOLIVIA:

- Lantana rugulosa* H.B.K. (Cocha-
bamba)

CHILE:

- Urbania egañioides* R. A. Phil. (Ta-
rapacá)

INDIA:

- Clerodendrum fragrans* var. *pleniflorum* Schau. (United Provinces)
Premna barbata Wall. (Punjab)

HAWAII:

- Lantana camara* var. *aculeata* (L.) Moldenke (Oahu)

SAMOA:

- Clerodendrum inerme* (L.) Gaertn. (Ofu, Tau, and Tutuila)
Clerodendrum speciosissimum Van Geert (Ofu and Tau)
Stachytarpheta urticaefolia (Salisb.) Sims (Tau)
Vitex trifolia var. *bicolor* (Willd.) Moldenke (Tau)

NIUE (SAVAGE) ISLAND:

- Clerodendrum inerme* (L.) Gaertn.
Clerodendrum speciosissimum Van Geert
Premna corymbosa (Burm. f.) Rottl. & Willd.
Premna taiensis var. *rimatarensis* F. H. Br.
Stachytarpheta urticaefolia (Salisb.) Sims
Verbena officinalis L.
Vitex trifolia var. *bicolor* (Willd.) Moldenke

CULTIVATED:

- Aloysia virgata* (Ruíz & Pav.) A. L. Juss. (Uruguay)
Citharexylum fruticosum var. *subvillosum* Moldenke (New York)
Clerodendrum bungei Steud. (Alabama)
Clerodendrum heterophyllum (Poir.) R. Br. (Australia)
Clerodendrum speciosissimum Van Geert (Niue Island)
Clerodendrum thomsonae Balf. f. (Niue Island)

Duranta repens L. (St. Bartholomew)

Duranta serratifolia (Griseb.) Kuntze (Uruguay)

Holmskioldia sanguinea Retz. (St. Bartholomew)

Lantana camara L. (Indiana)

Lantana camara var. *mista* (L.) L. H. Bailey (Uruguay)

Lantana foetida Rusby (Uruguay)

Stachytarpheta mutabilis var. *violacea* Moldenke (California)

Tectona grandis L. f. (Niue Island)

× *Verbena hybrida* Voss (St. Bartholomew)

Verbena peruviana (L.) Britton (St. Bartholomew)

× *Verbena wingei* Moldenke (England)*

Vitex agnus-castus L. (Oklahoma)

Lantana canescens var. *integrifolia* Moldenke, var. nov.

Haec varietas a forma typica speciei foliis integris recedit.—This variety differs from the typical form of the species in having its leaf-blades entire-margined. The type was collected by H. Garcia y Barriga (no. 10615) at La Vega on the road to Nocaima, alt. 950-1200 m., Cundinamarca, Colombia, between June 27 and 29, 1942, and is deposited in the United States National Herbarium at Washington.

Stachytarpheta cayennensis f. *albiflora* Moldenke, f. nov.

Haec forma a forma typica speciei corollis albis recedit.—This form differs from the typical form of the species in having white corollas. The type was collected by Dr. José Cuatrecasas (no. 14156) on the Pacific side of the Río Micay, alt. 5-20 m., Guayabal, El Cauca, Colombia, on February 26, 1943, and is deposited in the Britton Herbarium at the New York Botanical Garden.

Verbena variabilis Moldenke, sp. nov.

Herba perennis; caulibus leviter puberulo-strigillosis stramineis; ramis gracilimis plusminus puberulento-strigillosis; foliis sessilibus oppositis vel approximatis tripartitis revolutis utrinque strigillosis; spicis dense multifloris, rhachide dense patento-puberulo.

Perennial herb 50-100 cm. tall; stems tough, subterete or slightly tetragonal, lightly puberulent-strigillose, glabrescent toward the base, stramineous; branches short, very slender, more or less puberulent-strigillose; principal internodes 3-5 cm. long; leaves sessile, decussate-opposite or approximate, rather uniformly green on both surfaces, 2-3 cm. long, 1-2 cm. wide, the lower ones and those on the stems in general 3-parted with broad segments, each segment entire or with 1-3 large lobes, those on the branches 3-parted with very narrow, linear, pinnatisect segments, the lobes blunt at apex, all strigillose on both surfaces, revolute along the margins; peduncles slender, 2.5-9.5 cm. long, more or less strigillose-puberulent, especially toward the apex, usually with 1 or 2 pairs

of much reduced tripartite bracts near the apex and at the midpoint; spikes 2-5 cm. long, densely many-flowered; rachis densely spreading-puberulent; bractlets lanceolate, about 5 mm. long, long-acuminate, densely glandular-puberulent with erect spreading hairs; calyx about as long as the subtending bractlet, thin, lightly glandular-puberulent; corolla light-purple, white within, reddish-pink at base, its tube somewhat exerted from the calyx.

The type of this species was collected by R. D. Metcalf (no. 30255) in moist clay soil in good open drainage near Córdova, alt. 3050-3300 m., prov. Castrovirreina, Huancavelica, Peru, on March 27 or 28, 1942, and is deposited in the United States National Herbarium at Washington. The roots are used by the Indians against fleas.

× Verbena wingei Moldenke, nom. nov.

Verbena tenera × *Aubletia* Winge, Proc. Linn. Soc. London 150: 236. 1938.

NEW YORK BOTANICAL GARDEN
NEW YORK CITY, NEW YORK

FURTHER OBSERVATIONS ON THE TELEMORPHIC EFFECTS OF CERTAIN GROWTH-REGULATING SUBSTANCES¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 564

J. M. BEAL

Introduction

The formative influences induced in plants by a number of growth-regulating substances have been described by ZIMMERMAN (3, 4) and ZIMMERMAN and HITCHCOCK (5, and other papers). Some of the most active of these were certain of the substituted phenoxy compounds, application of which they found resulted in modification of the pattern of leaves, flowers, and fruits, and in changing the correlation phenomena of organs. Five of these compounds have been used in experiments described in the present paper, and five additional substances have also been tested on one of the experimental plants.

Because pronounced morphological responses are often incited or induced in plants at considerable distance from the point of application of the growth-regulating substance, the term "telemorphic effect" or response has been proposed to cover them (1). These previously described experiments have since been repeated and extended, and additional data have been obtained.

The use of Carbowax as a carrier for growth-regulating substances was recently described by MITCHELL and HAMNER (2), and in my later experiments both Carbowax 1500 and lanolin have been employed for this purpose. The results of ZIMMERMAN and of ZIMMERMAN and HITCHCOCK had shown strikingly different responses or effects

induced by certain of the substituted phenoxy compounds. The experimental data presented here show that the carrier in which a specific compound may be applied may also profoundly influence the character and degree of the response. Sweet pea (*Lathyrus oderatus*), African marigold (*Tagetes erecta*), and Red Kidney bean (*Phaseolus vulgaris*) have been employed as experimental plants.

Observations

SWEET PEA

Since the previous report (1) describing the telemorphic effects in the sweet pea resulting from application of 4-chlorophenoxyacetic acid in lanolin, another series of plants has been grown in the greenhouse in 4-inch pots containing fertile soil. Six to eight plants were grown in each pot, and all in a given pot received the same treatment. The chlorophenoxy compounds were made up in 1% and 0.25% mixtures, in both lanolin and Carbowax 1500, and applied as small pellets near the center of the upper surface of the two leaflets of the first well-developed foliage leaf. The plants were observed daily for 2 weeks or more, or until they died—as occurred in several instances before that length of time had elapsed.

2-CHLOROPHENOXYACETIC ACID.—This substance resulted in only slight epinasty and stem curvature, whether applied in lanolin or in Carbowax. Enlargement of roots did not result, but the total root development was less than in control plants. Nearly all plants were

¹ This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

dead at the end of 2 weeks when Carbowax was the carrier, but when lanolin was used little effect was observed, the plants being similar to untreated controls.

4-CHLOROPHENOXYACETIC ACID.—Striking differences in responses occurred when this acid was used. When

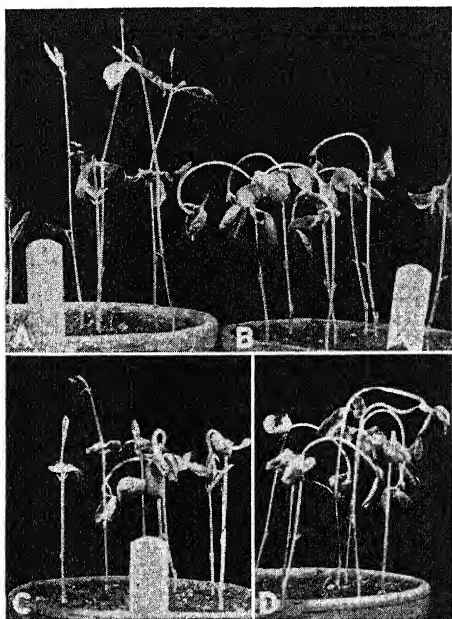


FIG. 1.—Sweet pea plants 48 hours after placing on first well-developed leaf a small pellet of: A, 1% mixture of 4-chlorophenoxyacetic acid in Carbowax; B, 1% mixture of same in lanolin; C, 1% 2,4-dichlorophenoxyacetic acid in Carbowax; D, 1% mixture of same in lanolin.

applied in Carbowax the leaves showed epinasty but the stems showed little or no curvature (fig. 1A). Both epinasty and stem curvature, however, quickly became evident when lanolin was the carrier (fig. 1B). But a more striking difference was in the effects produced on the roots of the treated plants. No visible enlargement was observed when a 1% mixture of the substance in Carbowax was used. A 0.25% mixture in Carbowax resulted in slight thickening

of roots on a portion of the plants. The roots of plants to which the same substance was applied, either as a 1% or a 0.25% mixture in lanolin, were often thickened for variable distances just back of the tips. The enlarged region varied from 1 mm. to 30 mm. in length and was usually two to three times the diameter of equivalent-aged roots from control plants at 8–10 days following treatment. Sections of these roots have again shown the same histological effects as those illustrated in the former paper (1). The plants began to die at 10 days; but, unlike those treated with 2-chlorophenoxyacetic acid, death occurred earlier when lanolin was the carrier. All the plants had died at 20 days after treatment with the 1% mixtures, but most of those treated with the weaker mixtures were still alive and growing after 4 weeks, although many of them had greatly enlarged stems.

2,4-DICHLOROPHENOXYACETIC ACID.—The plants to which this acid (fig. 1C, D) was applied reacted similarly, whether Carbowax or lanolin was the carrier, so far as epinasty and stem curvature were concerned. There was a difference in the responses of roots, however. The 2,4-dichlorophenoxyacetic acid as a 1% mixture in either carrier produced only a slight effect on the roots, and one not easily found; a 0.25% mixture in Carbowax also resulted in scarcely observable enlargement; and the same concentration in lanolin was nearly as effective as the 4-chlorophenoxyacetic acid in lanolin.

2,4,5-TRICHLOROPHENOXYACETIC ACID.—A 1% mixture in Carbowax resulted in slight root enlargement only, while a 1% mixture in lanolin, as well as 0.25% mixtures in both Carbowax and lanolin, resulted in pronounced root enlargement.

2,4,6-TRICHLOROPHENOXYACETIC ACID.

—A 1% mixture in lanolin resulted in scarcely visible responses on either leaves or stems, and these disappeared completely after 2–3 days. Growth then continued for a period of 2–3 weeks, when the terminal buds became yellowed, the leaf primordia thickened, and elongation ceased. The stem also thickened just back of the terminal bud, the entire plant began to lose its green color, and death followed shortly thereafter.

Each of the preceding phenoxy compounds resulted in noticeable stem swelling at various places on many of the plants, even though not applied directly to the stems. Only a few of the plants to which 2,4,5-trichlorophenoxyacetic acid was applied in lanolin developed adventitious roots, and these appeared almost entirely at and just above the ground line, never involving a length greater than 1 inch.

AFRICAN MARIGOLD

The African marigold is so sensitive and responds so readily to such a variety of stimuli that perhaps it is not a good experimental subject. Some of the results obtained in two series of experiments have been so striking, however, that a record of them seems warranted. The first series of plants was grown in the greenhouse during early spring, in flats containing fertile garden soil. When the first pair of true leaves had expanded the plants were treated by applying 1% lanolin mixtures of the compounds as a narrow ring around the first internode. Four of the chlorophenoxy compounds were used.

2-CHLOROPHENOXYACETIC ACID.—A few of the plants to which this acid was applied showed a slight swelling of the internode at the region of application after 3–4 days but practically no epinas-

ty or stem bending. The plants seemed to recover completely, showing no observable effects after a week, and during a period of 4 weeks of observation they developed essentially as did controls.

4-CHLOROPHENOXYACETIC ACID.—The plants to which this acid was applied showed much more evident effects. Conspicuous galls, which attained a diameter two to three times that of the stem above and below the region of applica-

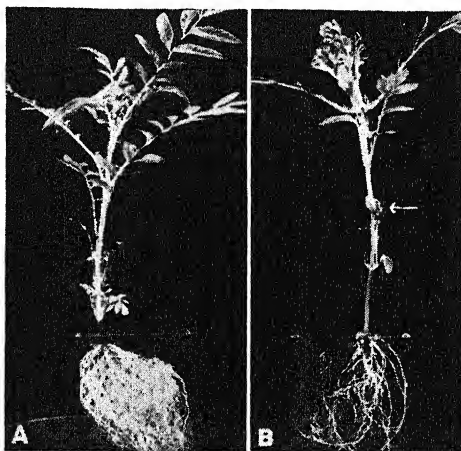


FIG. 2.—African marigold plants with roots in water cell: A, control, same age as B, plant at 5 weeks after ringing stem (shown by arrow) with 1% mixture of 4-chlorophenoxyacetic acid in lanolin.

tion, developed on all the treated plants (fig. 2B). There was marked suppression of root development as compared with controls (fig. 2A). The petioles of leaves distal to the place of application became much enlarged and yellowed and the terminal buds so distorted in form and structure after 2 weeks as to be no longer functional in further leaf production.

2,4,5-TRICHLOROPHENOXYACETIC ACID.—Application of this acid resulted in pronounced epinasty and stem curvature within a few hours. Within 48 hours the curvature was so great that in some plants the growing points were turned

directly downward. As growth was resumed, some plants assumed an S shape (fig. 3*B*), while others formed a complete loop. The leaves of these plants yellowed in 5-7 days following treatment, and all the plants were dead within 2 weeks.

2,4,6-TRICHLOROPHENOXYACETIC ACID.—No visible response occurred on either leaf or stem for approximately 2 weeks following application. Newly developing leaves appearing after 2 weeks

form of a fine mist with a small hand-operated atomizer.

2-CHLOROPHENOXYACETIC ACID.—A concentration of 0.13% resulted in pronounced epinasty and stem curvature at 24 and 48 hours following treatment, but the plants then seemed to recover gradually, so that at 96 hours there was little visible effect. Newly developed leaves and their leaflets were smaller and more slender than on control plants, and

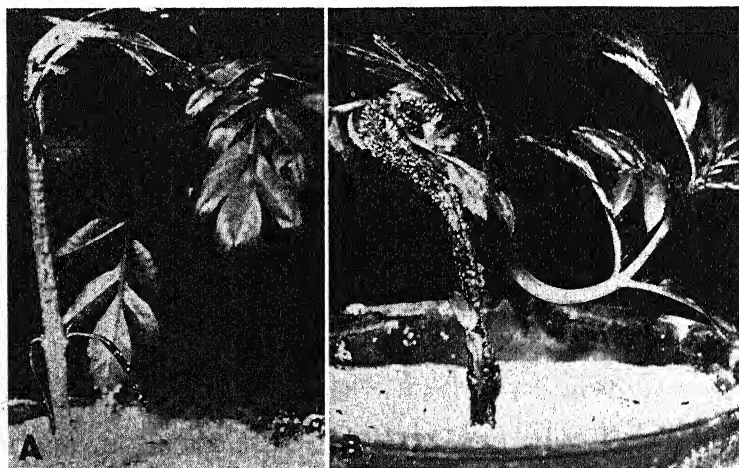


FIG. 3.—Marigold plants sprayed with: A, 0.06% aqueous solution of 2, 4-dichlorophenoxyacetic acid in 5% Carbowax (after 15 days); B, 0.06% aqueous solution of 2,4,5-trichlorophenoxyacetic acid in Carbowax (after 28 days).

were strongly attenuated and mottled. The terminal buds soon became malformed and no longer functional in further leaf development, and plants started dying 3-4 weeks following treatment.

A second series of plants was grown under similar conditions a little later in the spring, and when they had attained a height of approximately 10 inches they were treated by spraying on their leaves and stems the substituted phenoxy compounds dissolved in a 5% aqueous solution of Carbowax. About thirty plants were used for each of the compounds, and the mixtures were applied in the

their color was a paler green. Numerous small glistening protuberances became evident on the lower portions of the stems at 9-12 days, the epidermis and outer cortex split 5-7 days later, and roots emerged through the fissures. By now the terminal buds were distorted and no longer functional. Many plants were dead at 3 weeks and practically all of them were at 4 weeks following treatment.

4-CHLOROPHENOXYACETIC ACID.—A 0.06% mixture in 5% Carbowax resulted in both epinasty and stem curvature within 24 hours, followed by gradual

recovery, however, so that 4 days after treatment no effect was apparent. The plants continued apparently normal development for at least another week, after which the newly formed leaves were somewhat attenuated. The effect was not great enough to prevent continued growth, and these plants were vigorous and nearly normal in appearance 4 weeks after treatment.

2,4-DICHLOROPHENOXYACETIC ACID.
—Concentrations of both 0.06 and 0.13% resulted in marked epinasty and stem curvature within a few hours, followed by enlargement of the stems throughout most of their length after 3-4 days (fig. 3A). The epidermis of the swollen stems ruptured, the cortex often split longitudinally, and roots emerged—chiefly from the fissures—at about 3 weeks following treatment. The plants had now begun to lose their green color and quickly yellowed and died. Only two were living after 4 weeks, and they were dead 3 days later.

The plants reacted more quickly to the 0.13% than to the 0.06% mixture, and fewer plants produced adventitious roots, mainly because nearly half of them were dead 2 weeks after treatment. The responses were otherwise essentially alike for both concentrations.

2,4,5-TRICHLOROPHENOXYACETIC ACID.
—This was applied also as 0.06% and 0.13% mixtures in Carbowax. The effects were essentially like those produced by the same concentration of 2, 4-dichlorophenoxyacetic acid. Noticeable swelling, splitting of epidermis and cortex, and emergence of adventitious roots from the enlarged stems were conspicuous features (fig. 3B). Death did not occur quite so quickly following treatment with this acid as with the 2, 4-dichlorophenoxyacetic acid, but only one plant remained alive at 35 days.

BEAN

Three series of experiments, starting May 9, June 7, and September 30, respectively, dealing with the effects of the same five chlorophenoxy compounds have been carried out on the Red Kidney bean. In addition, indole-3-acetic acid, α -naphthaleneacetic acid, β -naphthoxyacetic acid, tryptophan, and naphthyl acetamide were applied to some of the plants in the last series. The chlorophenoxy compounds were applied as 1% and 0.25% mixtures in both Carbowax and lanolin, while the other substances were applied only as 1% mixtures in the two carriers.

The bean plants were grown in the greenhouse in flats containing fertile loam soil. Approximately thirty seeds were planted in each flat and after germination were thinned for uniformity and desired spacing, usually to twenty plants per flat. When the heart-shaped leaves had reached nearly full size (fig. 4B), a small pellet of one of the mixtures was applied to the upper surface near the base of both leaf blades of all the plants in a given flat. The mixture usually was placed in direct contact with the upper surface of the pulvinus, an effort being made to apply as nearly as possible equal volumes of the mixture to each leaf and to each plant. This volume has been estimated to average approximately 40 mg. per plant and to carry either 400 γ in the 1% mixtures or 100 γ in the 0.25% mixtures. There was some variation in the amounts applied to different plants, and there were differences in the responses made by individual plants, but not all the variations resulted from this cause. It is well known that differences in responses of individual plants to almost any type of stimulus occur, but in these experiments striking differences

in responses to or in the effects resulting from the application of a given compound have also been observed to depend on the carrier used.

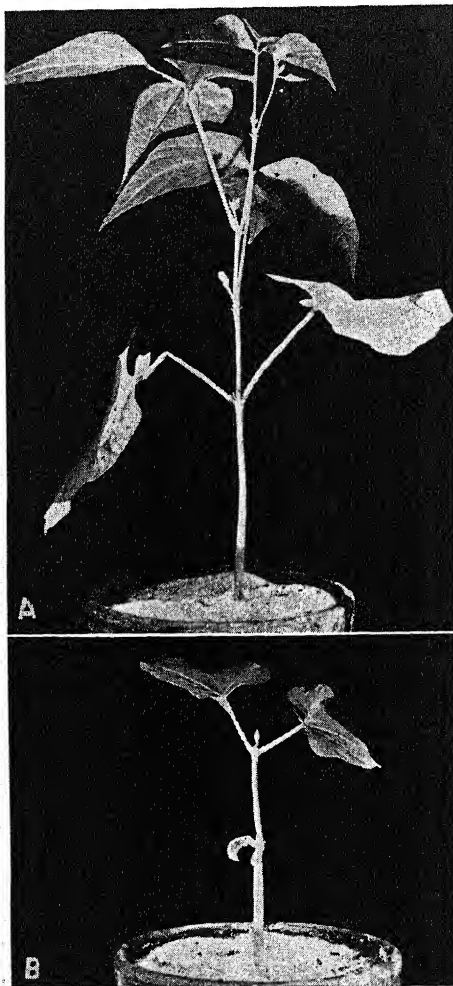


FIG. 4.—A, control plant of kidney bean, same age as those shown at 15 days after treatment; B, showing stage of development attained when mixtures were applied to bases of heart-shaped leaves.

2-CHLOROPHENOXYACETIC ACID.—A 1% mixture in Carbowax resulted in epinasty at 24 hours following application, with only about half the plants showing relatively slight stem curvature.

The leaves gradually recovered, but stem curvature increased during the next 2–3 days, producing a permanent bend in the first internode of nearly half the plants. Petioles of some of the heart-shaped leaves began to thicken in diameter on the third or fourth day, and the stems of many of the plants became swollen both above and below the second node (fig. 5A, B). The swelling occurred only above this node in some plants, only below it in others, and both above and below in still others. As a consequence of the increased diameter the epidermis often split and the cortex became fissured longitudinally for varying distances. On approximately one-third of the plants the heart-shaped leaves began to yellow and die at 7–8 days after treatment. These plants were usually dead by the end of 2 weeks. The terminal buds were killed on almost all the plants at 2 weeks, as were the axillary buds at more than half the first and second internodes. At 18–20 days after treatment most of the surviving axillary buds at the second node began to develop, followed 3–4 days later by some at the first node. Their development was slow, however, and small, narrow, and usually rolled leaflets developed. Their further expansion was slow, and on none of the plants observed over a period of 5 weeks were any of them more than one-third the size of the leaflets of control plants (fig. 4A). Most were misshapen and mottled, and none of the plants had flowered at 4 weeks. Several produced abundant adventitious roots at 5 weeks when grown under humid conditions (fig. 6), and a few plants flowered at approximately 5 weeks after treatment and formed viable seeds.

A 0.25% mixture in Carbowax resulted in similar but much less intense effects. Elongation of the second inter-

node and expansion of the first trifoliate leaf began at about 72 hours after treatment. The leaflets were attenuated and curled, much as with the 1% mixture, but they expanded somewhat more and were a much darker green than those of controls. In a few plants the second internodes, terminal buds, and petioles of the first trifoliate leaves showed light yellow glistening swellings after 7 days.

72 hours. The rate and character of growth seemed unaffected (fig. 5A, C), and blooming occurred at the same time as in controls. A 0.25% mixture in lanolin resulted in no visible effects. All plants treated with this acid in lanolin set pods as abundantly and as early as the controls.

4-CHLOROPHENOXYACETIC ACID.—A 1% mixture in Carbowax resulted in no

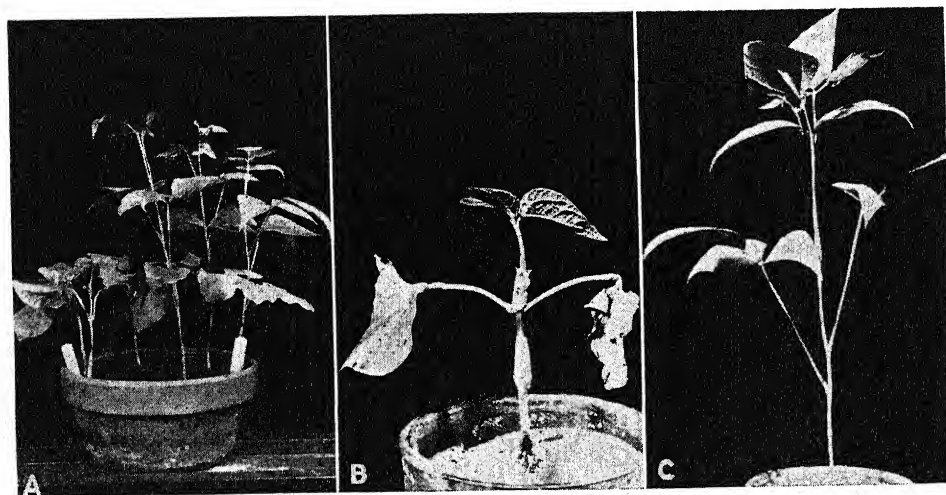


FIG. 5.—Effects of 1% mixture of 2-chlorophenoxyacetic acid on growth of bean: A, applied in Carbowax to three plants at left and in lanolin to three at right (after 12 days); B, effect in individual plant from Carbowax mixture after 21 days; C, same from lanolin mixture after same interval.

The second and third trifoliate leaves, evident at 14–17 days, showed even more distorted leaflets (fig. 12E). Axillary buds at the first and second internodes started expanding at 3 weeks, and their leaflets were also distorted. Flowers opened on four plants 20 days after treatment, but no pods had set 10 days later. None of the plants was killed by this treatment (fig. 6).

In striking contrast, the application of a 1% mixture in lanolin resulted in practically no visible effects. Slight epinasty was detectable at 24 hours, but this had disappeared completely before

visible effects for a period of approximately 48 hours. A few plants showed epinasty and slight stem curvature at 72 hours, but there was no apparent retardation of growth. The first trifoliate leaves came at the same time as on controls and appeared normal in all respects. Nearly half the second trifoliate leaves were definitely mottled and darker green than those of controls. All the third and subsequent leaves were also mottled (fig. 7A, B) but were dwarfed little or none. Flowering was not retarded nor was the setting of pods or seed development interfered with.

Plants treated with a 0.25% mixture in Carbowax showed slight epinasty at 24 hours, but this disappeared before 72 hours had elapsed. No stem curvature and no retardation of growth occurred.

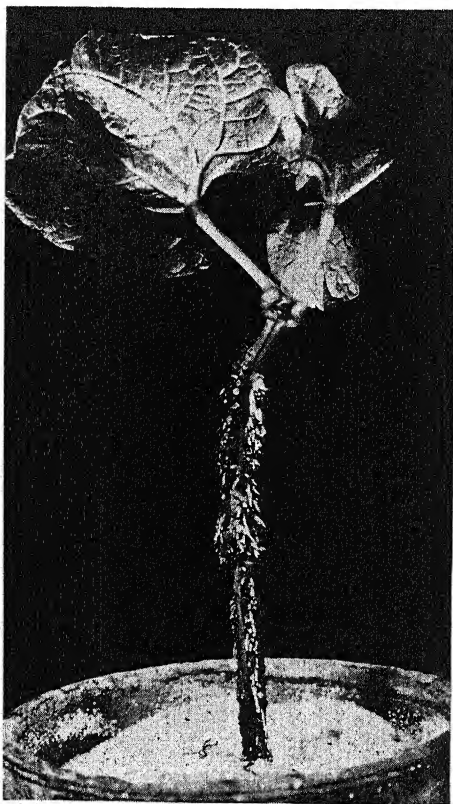


FIG. 6.—Effects of 0.25% mixture of 2-chlorophenoxyacetic acid in Carbowax 40 days after treatment. Both terminal and axillary buds killed; enlargement of hypocotyl and development of adventitious roots through essentially the entire length.

These plants developed apparently normally, except that they might possibly have grown slightly taller than the controls, but this may have been caused by some other factor or factors of the environment.

When lanolin was used as the carrier the responses were quicker and decidedly greater in degree than when Carbowax

was used, in marked contrast to the effects of 2-chlorophenoxyacetic acid in the same carriers.

A 1% mixture of 4-chlorophenoxyacetic acid in lanolin resulted in both epinasty and stem curvature 24 hours following application, the degree increasing gradually up to 72 hours. Terminal bud expansion was slowed little or none, but the leaflets of the first trifoliate leaf were markedly attenuated and rolled upward. The internodes appeared somewhat more elongated than those of controls, as did also the petioles of the trifoliate leaves. Only one plant showed any swelling of the terminal bud. The rolling and attenuation of the leaflets were conspicuous at 7–8 days following treatment, and the unrolling and expansion were slow. As the leaflets unrolled and expanded on the tenth to fourteenth days (fig. 7A, C), they were strongly mottled and much darker green than those of controls. Blooming was not delayed, but nearly all flowers were distorted and no pods had set 10 days after the first flowers had opened. Later-formed leaves generally remained small and narrow (fig. 12G).

The effects of a 0.25% mixture in lanolin were almost as marked as those resulting from the 1% mixture of the same acid. Growth of the terminal bud began as early as on untreated plants, but the leaflets of the first trifoliate leaf were strongly attenuated and curled. After 4–6 days they had expanded somewhat but were crinkled and mottled, with dark green splotches. Leaves developed from axillary buds were similar, and the whole aspect of these plants was like those treated with 0.25% 2-chlorophenoxyacetic acid in Carbowax. A typical leaf at 28 days is shown in figure 12H. The plants flowered at the same time as controls, but the flowers were malformed

and no pods had set at 35 days following treatment.

2,4-DICHLOROPHENOXYACETIC ACID.
—Application of a 1% mixture in Carbowax resulted in epinasty and stem curvature within 6 hours following treatment. At 24 hours the curvature was so great as to invert the growing points and heart-shaped leaves of nearly all plants. The petioles of a few leaves were beginning to swell, and after 48 hours petioles

second node and terminal bud, in some on the hypocotyl and terminal bud, in others on the first and second nodes and terminal buds, and in still others it was essentially throughout the plants. Nearly all terminal buds became so swollen and distorted as to be nonfunctional, and hence there was little growth of trifoliate leaves (fig. 8C). Half of all treated plants died within 2 weeks following treatment, but a few of the survivors began to re-

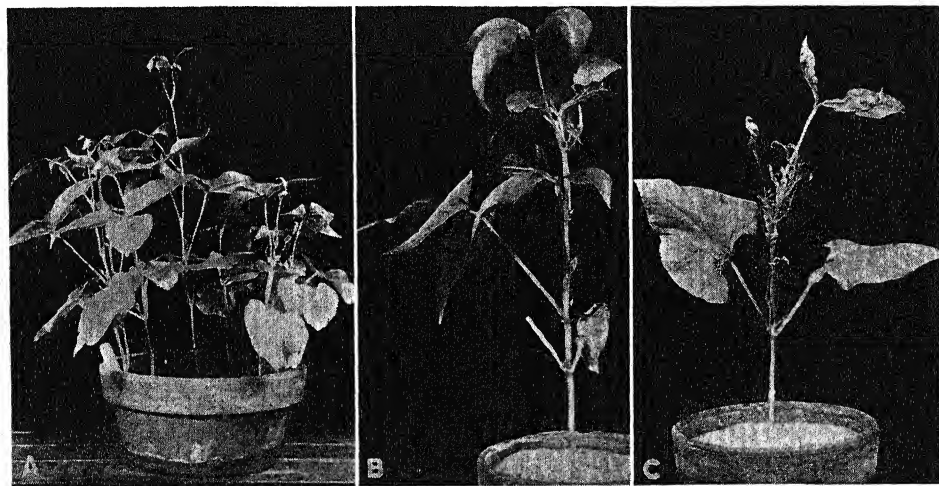


FIG. 7.—Effects of 1% mixture of 4-chlorophenoxyacetic acid on growth of bean: A, applied in Carbowax to four plants at left and in lanolin to three at right (after 12 days); B, effect on individual plant from Carbowax mixture after 21 days; C, same from lanolin mixture after same interval.

and second internodes of most plants were distinctly enlarged. Epinasty and stem curvature remained essentially unchanged during the following week. In the meantime, enlargements of the hypocotyls (principally near the ground line but at places above also) of the first internodes and of petioles continued. The heart-shaped leaves on about half the plants began to yellow, and six of eighteen plants had died 9 days after treatment. There was no uniformity among the plants as to the place or places at which telemorphic effects occurred; in some plants the enlargement was on the

cover. Three had functional terminal buds in the last series, and these began growing after about 16 days. The leaves produced were attenuated and strongly mottled, but one plant flowered on the twenty-fifth day after treatment. Pods were setting on the three plants at 35 days (fig. 8E).

A 0.25% mixture in Carbowax resulted in epinasty and stem curvature at 24 hours which persisted for 3–4 days. The terminal buds developed almost as quickly as those of controls but produced attenuated and mottled leaflets on only a part of the plants (fig. 8A). The leaflets

of the first trifoliate leaf were not rolled but were much darker green than those of the controls. The second and subse-

the cotyledonary node. On the whole these plants responded similarly, but somewhat less, than those treated with 0.25% 2-chlorophenoxyacetic acid in Carbowax or with 1% 4-chlorophenoxyacetic acid in lanolin. Blooming was not delayed, and fruit setting occurred—but at a reduced rate.

A 1% mixture applied in lanolin resulted in the same general effects as when applied in Carbowax. The swelling of hypocotyls, first and second internodes, petioles, and terminal buds paralleled closely in the two carriers, but with the telemorphic effects rather less severe when lanolin was the carrier (fig. 8D). The leaf bases and pulvini where the mixture was applied showed considerable enlargement at 8–9 days, with some of the heart-shaped leaves beginning to yellow. Two of twenty treated plants were dead after 2 weeks, and only three after 35 days. About half the surviving plants started recovering after 16–17 days, but their progress was slow (fig. 8F). The new leaves were small, slightly rolled, and generally mottled. Flowering was delayed for more than a week as compared with controls, but pods set abundantly.

A 0.25% mixture in lanolin had essentially the same effects as the same concentration in Carbowax (fig. 8B). In both instances the first trifoliate leaves were scarcely affected, while the second and later ones were dwarfed and mottled, with much darker green areas (fig. 12C).

2,4,5-TRICHLOROPHOXYACETIC ACID.

—A 1% mixture in Carbowax resulted in telemorphic effects strikingly similar to those resulting from application of a 1% mixture of 2,4-dichlorophenoxyacetic acid in the same carrier. Epinasty, stem curvature, stem and petiole swelling, retardation of normal growth, yellowing of heart-shaped leaves,

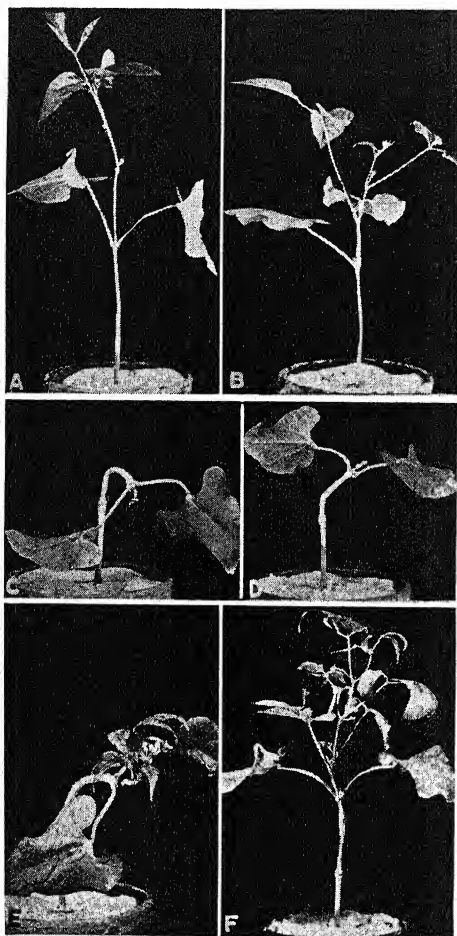


FIG. 8.—Effects of 2,4-dichlorophenoxyacetic acid on bean plants applied as: A, 0.25% mixture in Carbowax; B, same in lanolin (both after 15 days); C, 1% mixture in Carbowax; D, same in lanolin (both after 15 days); E, F, relative stages of recovery from 1% mixtures of this acid in Carbowax and lanolin, respectively, at 35 days following treatment.

quent trifoliate leaflets were still more attenuated, slightly rolled, and mottled (fig. 12D). Stem enlargement occurred in only a few plants and there only below

and death of treated plants were at essentially the same rate and degree from both substances. Only two plants remained alive 30 days after treatment, and they were decidedly yellowed and possessed no living buds. Neither trifoliolate leaves nor flowers were developed (fig. 9A).

A 0.25% mixture in Carbowax resulted in epinasty but no stem curvature. Terminal bud growth started almost as soon as in controls, but the rate of expansion of the trifoliolate leaves was much slower. Total swelling of stems and petioles was much less than in the 1% Carbowax mixture, although 6 days after treatment the hypocotyls on the majority of the plants showed enlargement at and above the ground line, with development of adventitious roots in this region. A small percentage of the plants showed enlargement of the hypocotyl, up to and including the cotyledonary node. Root primordia were generally evident at points along the swellings (fig. 9C). The base of the heart-shaped leaves, together with the pulvinus and sometimes the adjacent portion of the petiole, where the mixture was applied, were often yellowed and greatly enlarged. Many of these leaves gradually lost their chlorophyll and died at 18–21 days. Terminal bud growth was much retarded, but the trifoliolate leaves showed very little modification in form. A slight mottling was observed on the first-formed, but subsequent ones were nearly normal in appearance. Blooming was delayed a few days, but fruit setting was not apparently affected.

A 1% mixture in lanolin produced effects scarcely distinguishable from those just described, unless perhaps swelling of stems and slowing of growth were more pronounced (fig. 9B). None of the plants died from the treatment,

but most first trifoliolate leaflets were distorted and greener than controls. The later-formed leaves were normal in appearance.

A 0.25% mixture in lanolin produced effects similar to those described in both preceding lots. Growth was retarded at about the same rate, swellings as to position and degree were similar, and

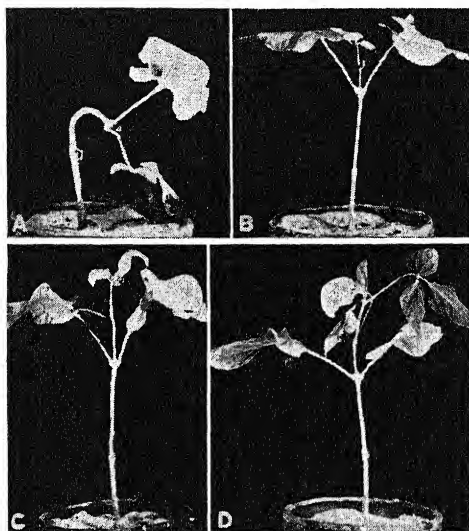


FIG. 9.—Effects of 2,4,5-trichlorophenoxyacetic acid on bean plants applied as: A, 1% mixture in Carbowax; B, 1% mixture in lanolin; C, 0.25% mixture in Carbowax; D, 0.25% mixture in lanolin. All at 15 days following application.

the trifoliolate leaves were mottled to about the same extent (fig. 9D). Subsequent development was similar in the three groups.

2,4,6-TRICHLOROPHENOXYACETIC ACID.

—A 1% mixture of this substance in Carbowax resulted in neither epinasty nor stem curvature. Development of the terminal buds was a little slower than on controls, and the first trifoliolate leaf showed darker green and mottled leaflets at 6 days. Subsequent trifoliolate leaves were smaller than the first and more strongly mottled, with darker green

areas, and were somewhat crinkled after 15 days (fig. 10A). Stems showed no effects, nor was blooming delayed. The plants set fruits as freely and as early as the controls, in spite of the mottled and smaller foliage leaves (fig. 12F).

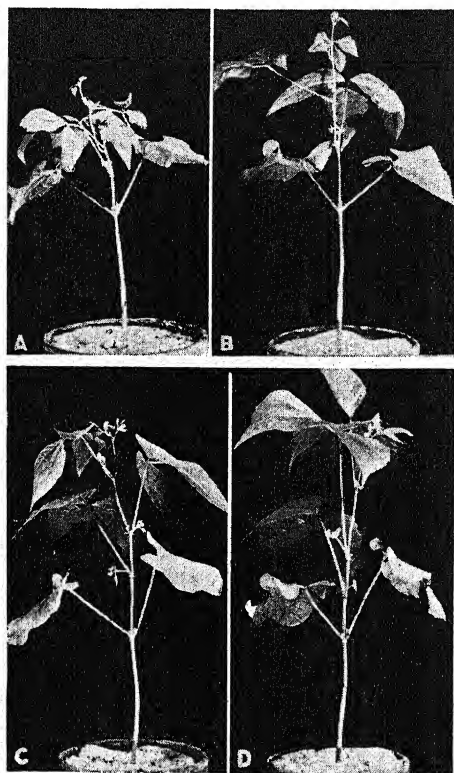


FIG. 10.—Effects of 2,4,6-trichlorophenoxyacetic acid on bean plants applied as: A, 1% mixture in Carbowax; B, 1% mixture in lanolin; C, 0.25% mixture in Carbowax; D, 0.25% mixture in lanolin. All at 15 days following treatment.

Plants treated with a 0.25% mixture in Carbowax showed no observable effects within 9–10 days, except that an occasional first trifoliate leaf showed a very slight amount of mottling. The second and subsequent trifoliate leaves were smaller, definitely mottled, and darker green than the first (fig. 10C), although leaves on one or two of the twen-

ty plants were apparently unaffected. Blooming and fruit setting were not delayed.

A 1% mixture in lanolin had no apparent effect during the first week following application. When the second trifoliate leaves began to expand, some of them showed slight mottling but no other effect (fig. 10B). Their subsequent growth and development appeared normal.

A 0.25% lanolin mixture apparently resulted in no visible effect on the plants to which it was applied. They developed in all respects as did the controls (fig. 10D).

TRYPTOPHAN, INDOLE-3-ACETIC ACID, β -NAPHTHOXYACETIC ACID, α -NAPHTHALENEACETIC ACID, AND NAPHTHYL ACETAMIDE.—Each of these substances was applied as a 1% mixture in both Carbowax and lanolin to the heart-shaped leaves of approximately twenty bean plants in the manner previously described for the phenoxy compounds. Except for moderate epinasty induced by the application of indoleacetic and naphthaleneacetic acids, none of the others—except the naphthoxyacetic acid—resulted in any apparent responses during the 5-week period the plants were under observation.

β -naphthoxyacetic acid resulted in slight epinasty and scarcely detectable mottling of the trifoliate leaves when lanolin was the carrier (fig. 12A). The leaflets were slightly greener than those of controls, but there was neither stem swelling nor retardation of growth or blooming (fig. 11B). The slight mottling disappeared, and the leaves quickly became normal in size and form.

When Carbowax was the carrier the degree of epinasty was essentially the same, but the amount of mottling of the trifoliate leaves was strikingly increased

(fig. 11A), giving the effect of a virus disease with small dark green areas inclosed by the pale yellow translucent veinlets (fig. 12B).

Discussion

Since MITCHELL and HAMNER have called attention to Carbowax as a carrier of growth-regulating substances and as a means of increasing their effectiveness as compared with water solutions,

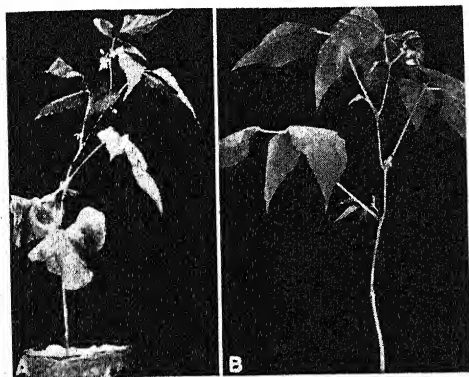


FIG. 11.—Effects of 1% mixtures of β -naphthoxyacetic acid on beans applied in: A, Carbowax, 22 days after application; B, lanolin, 28 days after application.

it seemed desirable to compare Carbowax with lanolin in similar tests.

From the detailed experiments carried out it is obvious that the effectiveness of a carrier depends upon the growth substance employed. For example, 2-chlorophenoxyacetic acid resulted in striking effects on the bean when applied either as a 1% or a 0.25% mixture in Carbowax, while the same concentrations in lanolin induced comparatively slight responses. In the case of the sweet pea the results were similar. Also, when 2,4,5-trichlorophenoxyacetic acid was dispersed as a 1% mixture in Carbowax such profound responses were induced in beans that all plants died in 3-4 weeks,

while the same concentration in lanolin showed its chief effects in the slowing of growth. A 1% mixture of naphthoxyacetic acid in Carbowax resulted in a conspicuous mottling of the first and second trifoliate leaves of the bean, and usually of the third; the fourth and later

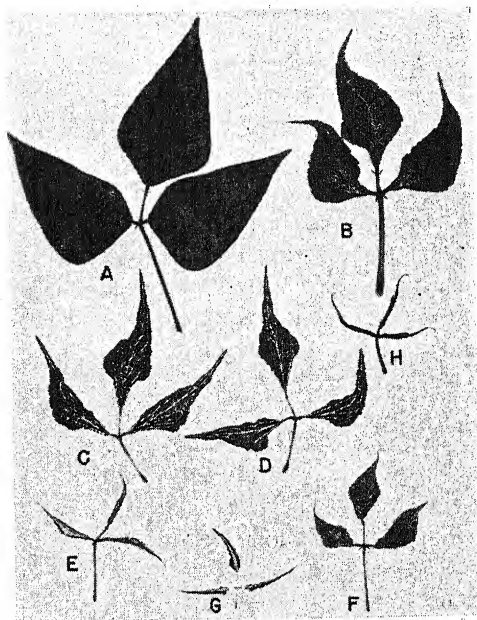


FIG. 12.—Trifoliate leaves of bean illustrating effects resulting from application of: A, 1% mixture of naphthoxyacetic acid in lanolin; B, 1% mixture of same in Carbowax; C, 0.25% mixture of 2,4-dichlorophenoxyacetic acid in lanolin; D, 0.25% mixture of same in Carbowax; E, 0.25% mixture of 2-chlorophenoxyacetic acid in Carbowax; F, 1% mixture of 2,4,6-trichlorophenoxyacetic acid in Carbowax; G, 1% mixture of 4-chlorophenoxyacetic acid in lanolin; H, 0.25% mixture of 4-chlorophenoxyacetic acid in lanolin. All 28 days after treatment—except B, which is 13 days.

ones were generally unaffected. The same concentration in lanolin resulted in no apparent effect on any of the leaves. On the other hand, when 2,4-dichlorophenoxyacetic acid was applied either to bean or to sweet pea, the responses were essentially the same whether Carbowax or lanolin was used as the carrier.

A striking difference in the responses occurred from the application of 4-chlorophenoxyacetic acid. When lanolin was used as the carrier with bean plants the responses were conspicuous. The growth rate was slowed; the trifoliolate leaves were attenuated and dwarfed and curled upward, and never expanded to full size. They were a much darker green color than leaves of controls. Both flowering and fruit setting were delayed. On the contrary, when this acid was applied in Carbowax practically no observable responses occurred until the appearance of the second or third trifoliolate leaves. Their leaflets were darker green in color than those of controls, and mottled, but flowering was not delayed nor was fruit setting reduced.

The strikingly different effects resulting from the application to plants of a specific growth-regulating substance, as well as from similar substances in the two carriers, are puzzling. How or in what form the substance enters the plant is not known. Does it enter by direct diffusion from the carrier or in combination with the carrier, as a solute in water associated with the carrier, as an ion of the acid, or does the substance become changed into another compound which is responsible for the reactions of the plant? Until answers can be supplied,

at least to some of these questions, it seems futile to indulge in much speculation. That these and similar experiments with growth-regulating substances are significant is beyond doubt, however, because of the possibility of establishing for them economic and practical applications as growth stimulators, growth depressors, or herbicides.

Summary

1. Five substituted phenoxy compounds have been applied to the sweet pea, African marigold, and Red Kidney bean in both Carbowax 1500 and lanolin as carriers. In addition, indole-3-acetic acid, α -naphthaleneacetic acid, β -naphthoxyacetic acid, tryptophan, and naphthyl acetamide were applied to the bean in both carriers.

2. The data indicate that the effectiveness of the carrier depends upon the growth-regulating substance employed. In these experiments, greater growth responses and form changes resulted, on the whole, when Carbowax was the carrier, although with some substances the responses were essentially equal with both carriers, and with one substance (4-chlorophenoxyacetic acid) the responses were much greater when lanolin was the carrier.

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CHLORINE ACCUMULATION IN DATE PALM VARIETIES

A. R. C. HAAS

Introduction

Among the many varieties of date palms grown in the Coachella Valley in southern California, the Deglet Noor is commercially the most important. Although certain diseases (1, 2) somewhat restrict its widespread use in some gardens, nevertheless this variety, because of the desirable qualities of its fruit, is being planted extensively.

Fruit quality may be the result of many factors, some of which do not lend themselves readily to exact measurement. An analysis of any differences in the chemical composition in the vegetative and reproductive phases of growth in the various varieties is therefore desirable, since chemical composition forms an excellent basis for estimating fruit quality.

This paper compares the total chlorine content of the pinnae and fruit from a great number of date varieties, including Deglet Noor. Samples were collected in several widely separated gardens, covering a considerable range of chloride concentration in the soil. Many of the varieties were also available in close proximity to one another in an individual garden in which the soil conditions for each variety were comparable. A comparison is also made of the chlorine content in date fruits (monocots) with that in citrus fruits (dicots)—both from orchards in low and in high chlorine soil areas.

Date palms are obtained as rooted offshoots from the parent palm, and hence the top and root are of the same variety. The tree is considered to be resistant to alkali salts (6), although it makes its best growth in the Coachella Valley in well-drained soils supplied with adequate quantities of irrigation water

of good quality. Because of its alkali tolerance, it is generally assumed that a certain amount of salt may be beneficial in the growth of the date palm, although data in this regard are still lacking.

The pinnae are highly siliceous, containing 9–25% of silica in the dry matter (2). The ash ranges from 12 to 29%, about 80% of which is silica (2).

Within the Deglet Noor variety, a remarkable uniformity was found (5) in the chemical composition of the fruit samples obtained from fertilizer plots. The determinations included: total soluble solids, insoluble residues, sugar, soluble solids not sugars, and total nitrogen (including nitrate nitrogen). The fertilizer appeared to influence only the average yield per palm.

It is known that the fruits of different varieties may differ in the type of sugar contained in the pulp; some—such as Halawy and Sayer—contain the invert type, whereas the Deglet Noor variety contains cane sugar (7).

The inorganic composition of date fruits (3) shows that the percentage of ash in the dry matter of the pulp of Deglet Noor is relatively low when compared with that of many of the other varieties. Because of the low ash values, it could not be determined whether chlorine also was low, since losses of chlorine are likely to occur in ashing unless special precautions are taken (4). The present results clarify the relation of chlorine with respect to the different date varieties.

Methods

The lowermost (oldest) leaf in which the pinnae were still in a healthy condi-

tion was selected. The pinnae were removed and wiped free of sand and dust. After drying in a ventilated oven at 65° C., they were finely ground in a Wiley mill and reheated at 65° C. just prior to weighing out the samples.

Large samples of several hundred mature fruits were collected except as noted.¹ The fruits were wiped free of dust after the removal of the calyx. The seed was then discarded, and the pulp finely cut to facilitate drying and placed in shallow glass dishes in a well-ventilated oven at 65° C. When dry, the pulp was allowed to cool and then finely ground in an electric coffee grinder, stored in tightly sealed glass jars, and reheated at 65° C. prior to weighing out the samples. If the dried pulp stood too long before grinding, the product became sticky and was difficult to handle; if too warm at the time of grinding the product resembled taffy. When properly cooled, the dried pulp was brittle and was easily ground.

All samples of pinnae or fruit pulp were weighed out in duplicate. Usually 50 gm. of finely ground dried pinnae or 40 gm. of ground dried fruit pulp was used for each chlorine determination.

Fifty ml. of a 5% solution of sodium carbonate was added to each porcelain dish (Champion sillimanite) containing the respective samples. Sufficient distilled water was added to wet the material thoroughly. The dishes were placed on a hot plate, and when warm the contents of each dish were stirred with a glass rod which was rinsed with distilled water and removed. This was followed by oven-drying until the material was ready for ignition. Care was exercised in the

ignition, since too high temperatures without sufficient sodium carbonate present is usually accompanied by losses of chlorine (4).

Fischer burners with closed vents were used for the ignitions. When thoroughly charred and cooled, the content of a dish was broken up with hot distilled water and filtered. Several further additions of hot distilled water were made, followed by filtration. The filter paper was added to the contents of the dish; and when dry on the hot plate, the ignition was repeated as previously described. Such ignitions at low heat were greatly aided by the washing-out of the soluble salts. After two or three ignitions, 10 ml. of 1:1 nitric acid was added to each dish, and both dish and filter paper were thoroughly washed with hot distilled water. The total filtrate (usually about 400 ml.) was placed in a beaker on a hot plate, an excess of silver nitrate (chlorine-free) gradually added with stirring, and when coagulated, the precipitate was filtered off and the washings with hot distilled water continued until the filtrate was free of silver. The filter paper and its contents were dried, the dry precipitate transferred to a glazed paper, and the filter paper ignited above the crucible, after which the precipitate was added. A few drops of HCl and of HNO₃ were then added. The final heating of the crucibles was carried on at a very low heat until the precipitate just began to melt. After cooling in a desiccator, the crucibles were weighed, the silver chloride determined, and the chlorine calculated. Closely agreeing duplicate tests were conducted for each sample of pinnae or fruit pulp.

Results

As shown in table 1, three gardens afforded most of the experimental ma-

¹ Assistance in securing leaf and fruit samples was generously given by Dr. D. E. BLISS, Mr. M. M. WINSLOW, Mr. R. W. NIXON, and those in charge in the various gardens.

TABLE 1
CHLORINE IN DRY MATTER OF PINNAE OF DATE PALMS

Garden	Variety	Palm	Date of sampling	Total chlorine in dry matter (%)
Jennings Postlethwaite Faries Haywood Anderson Hayes	Deglet Noor.....	R8E, P10	Dec. 27, 1929	0.130
	Deglet Noor.....	R2, P2	Nov. 27, 1929	0.167
	Deglet Noor.....	R7E, P6S	Dec. 27, 1929	0.219
	Deglet Noor.....	No. 19	Dec. 14, 1928	0.226
	Deglet Noor.....		Dec. 13, 1929	0.364
	Deglet Noor.....	22	Dec. 14, 1928	0.374
	Deglet Noor.....	6 — 100—20	Nov. 8, 1930	0.212
	Deglet Noor.....	25	" " "	0.224
	Barhee.....	14—86—22	" " "	0.309
	Kustawy.....	25 21—67—14	" " "	0.319
	Halawy.....	25 22—85x—46	" " "	0.347
	Zahidi.....	25 21—65—4	" " "	0.377
	Deglet Beida...	25 5—30—1	" " "	0.395
	Sayer.....	25 22—88—44	" " "	0.431
	Zahidi.....	25 21—65—4	" " "	0.436
Russel	Khadrawy.....	25 21—67—3	" " "	0.534
	Deglet Noor,....	Blk. 2, R8, P7	Oct. 6, 1930	0.104
	Maktoom.....	Blk. 2, R7, P8	" " "	0.111
	Halawy.....	Blk. 1, R2, P7	" " "	0.113
	Kustawy.....	Blk. 2, R1, P4	" " "	0.162
	Khadrawy.....	Blk. 2, R1, P8	" " "	0.202
	Zahidi.....	Blk. 2, R2, P5	" " "	0.202
	Hayany.....	Blk. 1, R1, P7	" " "	0.224
	Thoory.....	Blk. 2, R12, P7	" " "	0.261
	Barhee.....	Blk. 1, R2, P5	" " "	0.287
	Saidy.....	Blk. 1, R3, P7	" " "	0.373
	Iteema.....	Blk. 1, R10, P7	" " "	0.532
U.S.D.A.	Deglet Noor.....	R99, P5	Dec. 30, 1943	0.275
	Khadrawy.....	R100, P4	" " "	0.319
	Maktoom.....	R15, P12	Dec. 30, 1943	0.130
	Deglet Noor.....	R12, P4	" " "	0.152
	Hellali.....	R14, P12	" " "	0.247
	Dayri.....	R15, P6	" " "	0.260
	Barhee.....	R10, P12	" " "	0.262
	Halawy.....	R14, P4	" " "	0.298
	Zahidi.....	R9, P5	" " "	0.299
	Rhars.....	R12, P12	" " "	0.304
	Saidy.....	R10, P5	" " "	0.313
	Hayany.....	R13, P12	" " "	0.313
	Khalasa.....	R14, P11	" " "	0.331
	Medjool.....	R10, P11	" " "	0.335
	Iteema.....	R16, P11	" " "	0.337
Mecca	Khadrawy.....	R13, P4	" " "	0.386
	Kustawy.....	R16, P10	" " "	0.422
	Tazizoot.....	R16, P5	" " "	0.475
	Sayer.....	R15, P4	" " "	0.543
	Thoory.....	R11, P5	" " "	0.647

TABLE 2
CHLORINE IN DRY MATTER OF PULP (INCLUDING SKIN) OF DATE FRUITS

Garden	Sample no.	Variety	Palm	Date of sampling	Total chlorine in dry matter (%)	Total chlorine in average fruit (gm.)
Faries	39	Deglet Noor	R26, P14	Sept. 28, 1932	0.309	0.0216
Haywood	38	Deglet Noor		Sept. 28, 1932	0.173	0.0129
Haywood	105	Deglet Noor		Nov. 6, 1930	0.282	0.0227
Rosenberger	34	Deglet Noor			0.195*	0.0105*
Downing	45	Deglet Noor	R4, P9	Sept. 28, 1932	0.300*	0.0222*
Postlethwaite	46	Deglet Noor	R3, P4	Sept. 27, 1932	0.235*	0.0102*
Middleton	50	Deglet Noor	R1, P16	Sept. 27, 1932	0.375*	0.0196*
Postlethwaite	20	Deglet Noor		Sept. 24, 1930	0.535*	0.0256*
Gilette and Rosenberger	31	Deglet Noor		Oct. 6, 1930	0.450*	0.0343*
Postlethwaite	40	Menakher	R1, P2	Sept. 27, 1932	0.208	0.0211
Downing	37	Iteema	R2, P27	Sept. 28, 1932	0.352	0.0192
Downing	49	Tazizoot	R7, P27	Sept. 28, 1932	0.436	0.0258
Rosenberger	16	Saidy		Sept. 19, 1932	0.284	0.0210
U.S.D.A. at Indio	72	Deglet Noor (5)†		Sept. 30, 1943	0.158	0.0135
		Maktoom	R7, P8	Oct. 13, 1930	0.162	0.0124
		Deglet Noor (5)		Sept. 30, 1943	0.175	0.0137
	47	Deglet Noor	R8, P7	Oct. 6, 1930	0.248	0.0134
	38	Saidy	R3, P7	Oct. 6, 1930	0.262	0.0183
	37	Kustawy	R1, P4	Oct. 6, 1930	0.276	0.0125
	57	Thoory	R12, P7	Oct. 6, 1930	0.325	0.0178
	44	Zahidi	R2, P5	Oct. 6, 1930	0.352	0.0215
	41	Hayany	R1, P7	Oct. 6, 1930	0.443	0.0323
	50	Khdrawy	R1, P8	Oct. 6, 1930	0.476	0.0231
Russel Bros. tropical garden at Thermal	34	Deglet Noor	R77, P17	Sept. 20, 1932	0.218	0.0124
	28	Deglet Noor	R78, P2	Sept. 20, 1932	0.240	0.0172
	120	Barhee		Oct. 17, 1930	0.249	0.0182
	43	Deglet Noor	R78, P1	Sept. 28, 1932	0.290	0.0228
	25	Zahidi	R76, P6	Sept. 20, 1932	0.307	0.0182
	147	Kustawy		Oct. 17, 1930	0.314	0.0104
	19	Tafazwin	R77, P2	Sept. 20, 1932	0.319	0.0229
	129	Zahidi		Oct. 17, 1930	0.453	0.0265
	22	Halawy	R81, P2	Sept. 20, 1932	0.465	0.0194
	135	Khdrawy		Oct. 17, 1930	0.478	0.0269
	138	Halawy		Oct. 17, 1930	0.478	0.0237
	132	Deglet Beida		Oct. 17, 1930	0.562	0.0251
	141	Fard		Oct. 17, 1930	0.621	0.0379
	126	Ista Amran (Sayer)	22-88-44 25	Oct. 17, 1930	0.694	0.0482
Mecca experimental plots		Deglet Noor (6)	R12, P1	Sept. 29, 1943	0.227	0.0161
		Saidy (7)		Oct. 13, 1943	0.333	0.0290
		Halawy (8)	R14, P2	Sept. 1, 1943	0.337	0.0160
		Zahidi (6)	R9, P1	Sept. 1, 1943	0.351	0.0152
		Maktoom (10)		Oct. 13, 1943	0.378	0.0204
		Barhee (10)		Oct. 13, 1943	0.378	0.0226
		Deglet Noor (10)		Sept. 29, 1943	0.386	0.0241
		Khalasa (10)		Oct. 13, 1943	0.397	0.0227
		Deglet Noor (5)		Sept. 29, 1943	0.398	0.0243
		Thoory (8)	R11, P1	Sept. 1, 1943	0.422	0.0193
		Thoory (10)		Oct. 13, 1943	0.462	0.0191
		Dayri (8)	R15, P6	Sept. 29, 1943	0.470	0.0252
		Zahidi (9)		Oct. 13, 1943	0.525	0.0290
		Sayer (9)	R15, P1	Sept. 1, 1943	0.536	0.0207
		Saidy (7)	R10, P1	Sept. 29, 1943	0.557†	0.0249†
		Khdrawy (8)	R13, P1	Sept. 29, 1943	0.569	0.0330
		Tazizoot (8)	R16, P4	Sept. 1, 1943	0.618	0.0326
		Kustawy (6)	R16, P6	Sept. 1, 1943	0.628	0.0184
		Tazizoot (8)		Sept. 29, 1943	0.677	0.0402

* Decline-diseased.

† Nos. in parentheses indicate numbers of fruits.

‡ Immature fruit.

terial. Samples of soil² at the 6-12-inch depth were taken in the U.S.D.A. garden, in which a chlorine content of 22 p.p.m. of dry soil was found. At the Mecca plots the excessive chlorine in the soil was greatly reduced prior to planting of the date offshoots through the use of sulphur followed by leaching. At the 12-inch depth the soil in the Mecca plot recently showed only 40 p.p.m. chlorine.

irrigation water, as well as fertilization and other factors. The table indicates that, in the three principal locations in which many varieties were closely associated, the chlorine content in the dry matter in the pinnae of Deglet Noor is usually lower than in comparable samples from other varieties.

In table 2 the chlorine content is expressed as percentage of dry matter in

TABLE 3
CHLORINE IN PEEL AND PULP OF CITRUS FRUITS

FRUIT	LOCATION	DATE	No. OF FRUITS	CHLORINE IN DRY MATTER (%)	
				Peel	Pulp
Valencia orange. Valencia orange. Valencia orange. Lemon (tree-ripe).... Valencia orange. Lemon (tree-ripe).... Valencia orange. Valencia orange. Valencia orange. Lemon (tree-ripe)....	Low chlorine areas				
	Citrus Exp. Sta., Riverside (pa- thology plots)	July 11, 1942	16	0.039	0.044
		Aug. 12, 1942	14	.033	.045
		Oct. 9, 1942	14	.014	.027
		June 19, 1942	14	0.014	0.015
	High chlorine areas				
	Oxnard	June 19, 1942	8	0.016	0.025
		June 19, 1942	5	.029	.025
	La Habra	May 3, 1943	7	.025	.039
		May 3, 1943	8	.031	.040
		May 3, 1943	11036
		May 3, 1943	11	0.036	0.023

At this same depth in the omphalia root-rot area (1) in the Russel garden, the soil contained 758 p.p.m. of chlorine, and it was here that collection of material from many palm varieties was made.

Deglet Noor pinnae vary considerably in their chlorine content, depending on the garden from which they are collected (table 1). This probably takes into account the chlorine status of the soil and

the fruit pulp (including the skin). Variations occur in the percentage of chlorine in the pulp of Deglet Noor fruits collected in various gardens. Increased chlorine content in decline-diseased fruits possibly is related to the low dry matter content of such material.

In the dry matter of the pulp, Deglet Noor generally shows less chlorine than in comparable portions of fruits of other varieties grown in the same garden.

Since complete records were kept of the number of fruits collected and their

² Assistance in obtaining these soil samples and in their analyses was kindly given by Dr. D. E. BLISS and Mr. B. M. LAURANCE.

fresh and dry weights, it was possible to calculate the total chlorine in an average fruit. The size of the fruit should also have been taken into account, but these data were not available. Even without this latter factor, the data in table 2 indicate the low chlorine content per fruit in Deglet Noor, especially in the data for the U.S.D.A. garden. The data for the Deglet Noor fruits from various gardens suggest a fairly wide variation in the chlorine content in the dry matter of the pulp within the same variety in various gardens.

The percentage of chlorine in the dry matter of the pulp of date fruits, although very low in all varieties, is nevertheless high when compared with that in citrus (table 3), for in date fruits (monocots) the percentage may be ten times that found in citrus fruits (dicots).

Summary

1. The chlorine content in the dry matter in the pinnae of the Deglet Noor variety is usually lower than in comparable samples in other date varieties.
2. Variations occur in the percentage of chlorine found in the pulp of Deglet Noor fruits collected in various gardens.
3. When collected in the same garden, the dry matter of the pulp of the Deglet Noor variety generally contains a lower percentage of chlorine than is found in comparable portions of fruits of other varieties.
4. Although the chlorine content in the dry matter of date pulp may appear to be small, it is about ten times that found in citrus fruits in orchards in high chlorine areas.

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TOXIC SUBSTANCES FROM THE CULTURE MEDIA OF GUAYULE WHICH MAY INHIBIT GROWTH

JAMES BONNER AND ARTHUR W. GALSTON

Introduction

The question of whether or not plants may emanate substances which accumulate in the soil and which may be toxic to and inhibit growth of the same or of other species is at present a controversial one. Early in the present century, PICKERING (8) suggested that an inhibitory effect of grass on the growth of apple trees, observed by him, might be due to toxic materials derived from the grass. This work was continued (9), and the results seemed to show that in numerous combinations one species might deleteriously affect the growth of a second. More concrete evidence bearing on the possibility of toxic substances in the soil was shown by the work of SCHREINER and co-workers (12-18). Four compounds—picolinic acid, dihydroxystearic acid, salicylaldehyde, and vanillin—isolated from soils were found to be very toxic to wheat seedlings. The infertility of certain soils appeared to be correlated with the presence of one or more of these compounds. Most soil infertility, however, is now known to be associated with conditions other than the accumulation of one of these four substances.

Injurious effects of black walnut trees on other plants have been reported (6, 11), and DAVIS (4) has sought to identify the toxic principle with juglone (5-hydroxy 4 α -naphthoquinone), which is found in the black walnut tree itself. Although juglone was shown to be toxic to alfalfa and to tomato, there was no evidence that this substance is actually released into the soil, or that it is in fact a toxic agent under natural conditions.

PROEBSTING and GILMORE (10) have investigated the difficulty of re-establishing peach trees in old peach orchards. After demonstrating that this phenomenon was due neither to nutrient deficiencies nor to pathogenic organisms, they found that incorporation of peach roots or alcohol extracts of peach roots in soil resulted in the appearance of toxicity symptoms in young trees planted in such soil. BENEDICT (2) also showed that dried roots of bromegrass are inhibitory to the growth of the same species and suggested that its living roots may also excrete substances toxic to the plant. BENEDICT suggests that the thinning out of bromegrass stands may be the result of the accumulation of a toxic substance from the roots. The data of MYERS and ANDERSON (7) support the contention of BENEDICT.

In the experiments on growth inhibition by dead roots or root extracts, evidence is lacking that the toxic substance is in fact given off by the roots of the living plant, or is in fact the agent responsible for growth-inhibition responses in the soil or other substratum. It might also be objected that substances extracted from dead root material are not necessarily present in the living plant. In the work reported here these objections have been met to some extent. Organic substances inhibitory to the growth of plants of guayule were obtained from nutrient solution recirculated through cultures of healthy growing guayule plants. Inhibitory substances of similar chemical properties were isolated in pure form and chemically iden-

tified from water in which guayule roots had been allowed to stand briefly. The experiments were undertaken in February, 1941.

MATERIAL AND METHODS.—Guayule plants of the strain no. 593 were used throughout. Seeds were supplied by the American Rubber Producers during 1941, and thereafter by the Special Guayule Research Project, U.S. Bureau of Plant Industry, Soils, and Agricultural Engineering. The seeds were sown in washed river sand and germinated in the greenhouse at 70°–80° F. The seedlings were transplanted to 4-mesh gravel contained either in 2-gallon glazed crocks or in 1- or 2-gallon cans which had been previously painted with a nontoxic asphaltum paint. In some cases nursery plants supplied by the Guayule Project were used. Plants were supplied daily (in some cases three times a week) with Hoagland's nutrient solution. This solution has previously been shown (3) to be optimal or near optimal for the growth of guayule. In none of the experiments concerning inhibition carried out over a period of 3½ years did any recognizable symptoms of mineral deficiency occur.

Experimentation

LEACHING.—That substances or conditions unfavorable to the growth of guayule accumulate in containers in which the plants are actively growing is shown by the following experiment. Small nursery plants were planted in 4-mesh gravel contained in 1-gallon cans which were provided with abundant drainage and placed on a well-drained, well-lighted greenhouse bench. After 1 week, new growth began to appear on the (topped) plants, and the experiment proper was begun. Eighty containers of plants selected for uniformity were disposed in rows of four along a greenhouse

bench. The plants of alternate rows served as controls and were supplied three times per week with Hoagland's solution, 250 ml. per container per application. On the remaining days of the week the containers were flushed with water. The experimental rows were supplied with nutrient solution and with water which had been leached through 4-mesh gravel in which other guayule plants were grown. To this end, vigorous fast-growing plants in 2-gallon cans were used. Three times each week nutrient solution was poured through the containers of each plant until 1 liter of leached nutrient had been collected from each. The nutrient so collected was found by analysis to have undergone no significant reduction in either nitrate or phosphate concentration. Leached nutrient was dispensed to the experimental plants at the rate of 250 ml. per container three times per week. The water with which the experimental plants were supplied was similarly leached. The containers of the donor plants (from which the leachings were obtained) were periodically flushed with water, which contained only negligible amounts of salts.

After 6 weeks of growth, those plants which received leached water and nutrient were visibly smaller than the controls. Measurements of total heights of the plants at this time are given in table 1. Two months after initiation of the experiment, all the plants were measured, harvested, dried, and weighed. Table 1 gives the data on the total dry weight per plant found in the two treatments. Plants which received leached water and nutrient were significantly retarded, both as to growth in height and as to dry-weight accumulation, in comparison with plants which received fresh water and fresh nutrient. The retarded plants of this experiment were otherwise

healthy in appearance and differed from the controls in no marked way other than in size.

This type of experiment was repeated seven times. In six cases results similar to those of table 1 were obtained; in the seventh case no retardation of growth was obtained. The cause of this discrepancy may lie in the fact that the experi-

planted to river sand contained in 4-inch clay pots. All plants were given nutrient on alternate days. On the intervening days the controls were watered with tap water, while the experimental plants were watered with tap water which had been leached through gravel containing actively growing guayule plants. Conversely, leachings from tomato plants

TABLE 1

GROWTH OF GUAYULE PLANTS IN GRAVEL CULTURE AND SUPPLIED EITHER WITH NUTRIENT AND WATER OR WITH NUTRIENT AND WATER PREVIOUSLY LEACHED THROUGH OTHER GUAYULE GRAVEL CULTURES. HEIGHT MEASUREMENTS AFTER 6 WEEKS; DRY-WEIGHT MEASUREMENTS AFTER 2 MONTHS. EACH FIGURE IS MEAN OF FOUR PLANTS

SOLUTION	PLOT NUMBER										Av.
	1	2	3	4	5	6	7	8	9	10	
Control (not leached nutrient, etc.) Leached nutrient	Height per plant (cm.)										
	22.7	21.3	21.3	21.8	22.0	21.5	21.8	21.0	21.0	22.0	21.6±0.17
	16.2	19.0	15.2	18.0	15.2	14.0	13.2	15.2	16.8	18.2	16.1±0.60*
	Dry weight per plant (gm.)										
Control (not leached nutrient, etc.) Leached nutrient	4.70	4.45	4.15	4.65	4.48	4.27	3.68	4.65	4.42	4.48	4.39±0.096
	2.05	2.73	1.90	2.20	2.12	2.18	1.77	2.18	1.90	2.65	2.17±0.098*

* Difference between this value and that of control significant at 1% level.

ment in question was carried out during the winter months, when growth-inhibition effects were found to be in general less than in the spring and summer. Results generally similar to those of table 1 were also obtained when only the water and not the nutrient solution was leached.

A retarding effect of guayule root leachings was not found in either of two experiments in which this material was applied to tomato plants. In the experiments of table 2, tomato plants 3 weeks old from the time of planting were trans-

did not appear to retard the growth of guayule (table 3). In these experiments, vigorous gravel-grown tomato plants approximately 3 feet tall were leached as described and the leached solution applied to young guayule plants. The data of tables 2 and 3 suggest that in the retarding effect of guayule root leachings on the growth of guayule plants there may be a degree of specificity.

RECIRCULATION.—In this type of experiment nutrient solution was allowed to drip, at the rate of about 500 cc. per

hour, on crocks containing gravel cultures of guayule plants 6-12 months old. The nutrient as it drained from each crock was led into a second crock containing similar gravel and three guayule seedlings. The nutrient flowing from the

plants, which in the case of the experiment of table 4 were 8 months old. Both lots of nutrient were adjusted at weekly intervals so that the concentration remained relatively constant. Table 4 gives data from an experiment of this

TABLE 2

GROWTH OF TOMATO PLANTS IN SAND CULTURE AND SUPPLIED EITHER WITH WATER OR WITH WATER LEACHED THROUGH GRAVEL CONTAINING ACTIVELY GROWING GUAYULE PLANTS. EXPERIMENT G-9 CONTINUED FOR 2 WEEKS; EXPERIMENT G-5 FOR 4 WEEKS

SOLUTION	EXPERIMENT G-9		EXPERIMENT G-5	
	No. of plants	Dry weight per plant (gm.)	No. of plants	Dry weight per plant (gm.)
Control (water not leached).....	27	0.790 ± 0.029	10	1.19 ± 0.064
Water previously leached through cultures of guayule.....	18	0.787 ± 0.028	10	1.03 ± 0.061

TABLE 3

GROWTH OF GUAYULE PLANTS IN GRAVEL CULTURE AND SUPPLIED EITHER WITH WATER OR WITH WATER LEACHED THROUGH GRAVEL OR THROUGH GRAVEL CONTAINING ACTIVELY GROWING TOMATO PLANT. MEASUREMENTS OF HEIGHT AFTER 3 MONTHS. NONLEACHED NUTRIENT APPLIED THROUGHOUT

SOLUTION	EXPERIMENT G-17		EXPERIMENT G-18	
	No. of plants	Height (cm.)	No. of plants	Height (cm.)
Control (water not leached).....	16	11.31 ± 0.89	16	8.25 ± 0.55
Water previously leached through 4-mesh gravel.....	16	10.32 ± 0.63	16	10.00 ± 0.47
Water previously leached through gravel culture of tomato plants....	16	11.78 ± 0.99	16	8.74 ± 0.92

crock of seedlings was collected and recirculated through the system. Control seedlings were grown in gravel in crocks provided with a nutrient-recirculating system in which no older plants were included. Thus the control plants were merely grown in recirculated nutrient, while the experimental plants were grown in nutrient recirculated through both the seedling cultures and the older

type. The seedlings whose nutrient was recirculated over older plants appeared inhibited in growth as compared with those whose nutrient was recirculated over only the seedlings themselves. No deficiency symptoms appeared in either group throughout the experiment, and the plants of the two groups appeared to differ in no marked way other than in size. The results of the experiment of

table 4 were confirmed in a second experiment. In two further tests no retardation of growth appeared in the experimental plants. These discrepant results were correlated with the fact that the large plants used in the recirculation systems of the latter two experiments did not grow actively and later were found to be severely pot-bound.

GROWTH OF SEEDLINGS UNDER OLDER PLANTS.—Guayule seedlings were planted under older, established guayule plants growing in crocks filled with 4-mesh gravel. The lower branches of the large plants were trimmed away to avoid excessive shading of the seedlings. Water and nutrient was supplied several times a day in order to minimize water and nutrient competition between the older plant and the seedling. Controls were planted in similar gravel not containing older plants. In three experiments of this kind it was found that seedlings 1.5 cm. tall, when planted under older plants, either died or were greatly retarded in growth in comparison with similar seedlings not so planted. Interpretation of this result was made uncertain, however, because of the shading and possible competitive effects due to the older plants. The experiment was therefore modified as follows. A glass container of about 500-cc. capacity and provided with drainage at the bottom was sunk in the gravel which surrounded a 5-9-months old plant cultured in a 2-gallon crock. The small container was then filled with fresh gravel. Four seedlings were planted under each large plant, two being in the glass jar. Both sets of seedlings were therefore shaded by the larger plant, but two seedlings of each lot of four were subject in addition to any influences exerted on their growth by the culture medium of the older plant. Table 5 gives the results of two experiments of this

kind. In both cases, seedlings planted in jars under the older plant made more growth than the seedlings planted directly in the gravel surrounding the older plants. These two experiments indicate again that some unfavorable substance may emanate from an older guayule plant.

ASSAY FOR TOXIC ACTIVITY.—Various methods of assaying the toxic effect of solutions leached through guayule cultures were attempted. The most convenient and useful method was based on

TABLE 4

GROWTH OF GUAYULE SEEDLINGS AFFECTED BY RECIRCULATION OF NUTRIENT SOLUTION; EXPERIMENT G-37. MEASUREMENTS AFTER 3 MONTHS. EIGHTEEN PLANTS PER TREATMENT

Nutrient recirculated	Height (cm./plant)	Dry weight (gm./plant)
Over seedlings only. . .	24.3 ± 0.70	3.45
Over both seedlings and 8-months-old plants.	17.1 ± 1.76*	1.29

* Difference between this value and that of control significant at 1% level.

the fact that guayule seedlings make a rapid and luxuriant growth in unaerated solution culture. Seedlings 3-6 weeks old were transplanted individually to shell vials of 20-cc. capacity. Wooden boxes were constructed to hold the vials in such a way that each vial fitted closely into a hole in the top of the box, with only the apical 2-3 mm. of the vial protruding. In this way the roots of the plants were kept in darkness. Twenty vials were contained in each box. The plants were held in the vials in slits made in cork stoppers, a small piece of cotton holding each plant firmly in place. Each cork had in addition a small central hole for supplying distilled water to replace that lost in transpiration. Initial measurements of height were made on each

plant, and dry weights were obtained by sampling each lot. The seedlings were then allowed to grow in the greenhouse at a constant temperature of 80° F. for 2 weeks, at which time final heights and dry weights were determined. During the 2 weeks, the test plants grew an average of 3-10 mm. in height, depending on the nature of the initial planting material. Dry weight increased during the same time from an initial 7-10 mg. per plant to a final 20-30 mg. per plant.

was used as a substrate for the seedling plants unless otherwise noted. This concentration gave faster growth than lower concentrations and was somewhat superior to full strength. Although the pH of the nutrient had no influence over the range pH 5.0-7.0, all solutions were nevertheless adjusted to pH 5.5 before use. Fractions to be tested for toxic activity were always tested in a series of concentrations, each concentration being three times less than the preceding.

TABLE 5

EFFECT OF 5- OR 9-MONTHS-OLD GUAYULE PLANTS ON GROWTH OF SEEDLING GUAYULE PLANTS IN SAME CROCK. IN EXPERIMENT G-36 THE LARGE PLANTS WERE 5 MONTHS OLD; IN EXPERIMENT G-40, 9 MONTHS OLD

SEEDLING PLANTS	EXPERIMENT G-36				EXPERIMENT G-40		
	No. of plants	No. surviving	Height after 5 weeks (cm.)	Dry weight (gm./plant)	No. of plants	No. surviving	Height after 6 weeks (cm.)
In gravel contained in separate jars under large plants	8	7	6.5 ± 0.86	0.28 ± 0.044	40	26	5.15 ± 0.376
In gravel under large plants.....	8	7	3.1 ± 0.44*	0.084 ± 0.0058*	40	7	2.15 ± 0.266*

* Difference between this value and that of control significant at 1% level.

Table 6 gives data on the growth (in height and dry weight) of replicate lots of guayule plants. It may be seen that for growth in height the means from groups of ten plants vary on the average approximately 7% from the over-all mean. The data indicate that a difference of 30% or more in height between two groups of ten plants should be significant at the 5% level, while a difference of 41% or more should be significant at the 1% level. Quantitatively similar results were obtained in two other homogeneity tests. In general, differences in height growth of 50% or more may be safely taken as significant in the present assay. Hoagland's solution, diluted four times,

Each concentration was in turn tested on ten plants, and for every three sets of ten test plants one set of ten controls was included. As many as 550 plants were used simultaneously in one experiment. In all, more than seventy separate experiments have been carried out and approximately 1000 fractions assayed for toxic activity.

TOXICITY OF RECIRCULATED NUTRIENT SOLUTION.—In order to obtain large amounts of leached nutrient solution, an apparatus was set up in which 5 gallons of one-half strength Hoagland's solution was distributed so that it dripped through twenty 2-gallon crocks, each containing a guayule plant 6-8 months

old and 20 cm. or more tall. The nutrient solution was collected from each crock and recirculated twice each day. Facilities for the simultaneous recirculation of 60 gallons of nutrient solution were arranged. In general, the recirculation was continued for approximately 30 days. If during the 30 days any of the plants assumed an unhealthy appearance, it was

circulated nutrient were inhibited in growth as compared with control plants in fresh nutrient. In the mean, the recirculated nutrient supported only about 50% as much growth as the fresh nutrient. The seedling plants grown in the recirculated nutrient received adequate nitrogen and phosphorus from this source, as shown by analysis, and dem-

TABLE 6

GROWTH (MM./PLANT) IN HEIGHT AND DRY WEIGHT IN REPLICATE LOTS OF SEEDLING GUAYULE PLANTS IN NON-AERATED SOLUTION CULTURE. TWO-WEEK GROWTH PERIOD. INITIAL DRY WEIGHT, 70 MG./10 PLANTS

PLANT	PLOT NUMBER									
	1	2	3	4	5	6	7*	8*	9*	10
1.....	15	8	9	7	8	8	11	8	7	3
2.....	4	6	5	10	5	8	6	6	5	3
3.....	8	8	6	7	5	9	5	4	8	7
4.....	7	6	5	9	10	10	7	7	4	8
5.....	8	6	9	15	6	9	9	10	14	5
6.....	3	6	7	11	6	8	8	11	6	9
7.....	8	7	6	12	10	5	4	7	10	9
8.....	7	9	11	8	11	9	11	8	8	10
9.....	7	5	5	5	7	6	9	8	9	6
10.....	6	10	2	10	9	10	8	7	9	11
Average.....	7.3± 1.01	7.1± 0.50	6.5± 0.82	9.4± 0.91	7.7± 0.70	8.2± 0.51	7.8± 0.74	7.6± 0.62	8.0± 0.89	7.1± 0.89
Final dry weight (mg. per 10 plants).....	240	230	200	270	190	220	210	220	220	220
Increase in dry weight per 10 plants.....	170	160	130	200	120	150	140	150	150	150

* Received nutrient containing inactive fraction.

immediately replaced. Every precaution was also taken to avoid the presence of algae in the crocks and other parts of the recirculation system. This system occupied one entire greenhouse and necessitated a great deal of care.

In table 7 the results of the application of six such recirculated nutrient solutions to guayule seedlings are given. In these experiments, one-half strength Hoagland's solution was used for the control plants to simplify comparison with those grown in the recirculated one-half strength nutrient. Plants grown in re-

onstrated no visible deficiency symptoms. That the relatively poor growth supported by the recirculated nutrient is actually due to a toxic substance or substances will be demonstrated in the next section.

TOXIC SUBSTANCES IN RECIRCULATED NUTRIENT.¹—Preliminary experiments showed that recirculated solution could be concentrated to dryness without loss of its toxicity for guayule seedlings. Ashing the dried material resulted in

¹ Preliminary work on this subject was carried out in co-operation with Dr. SIDNEY GOTTLIEB.

total loss of the toxic activity, however, indicating that the toxic activity might be due to organic material present in the recirculated nutrient. Attempts were made to extract this toxic material from the dried recirculate with organic solvents. The activity could be removed with acetone, methyl or ethyl alcohol, or by ether after acidification of the dry material. Extraction with ether from non-

tivity passed into the alkali, leaving an inactive resinous residue. Addition of acid to the alkali extract separated the toxic activity into two fractions, one insoluble in acid, which precipitated out, and one which remained in solution. At most, 75 mg. of alkali-soluble acid-precipitable material (fraction 4a of table 9) could be obtained from the 30-day recirculate of 240 plants. This scar-

TABLE 7

GROWTH OF GUAYULE SEEDLINGS IN NUTRIENT SOLUTION PREVIOUSLY LEACHED THROUGH CULTURES OF GUAYULE PLANTS. EACH SOLUTION ASSAYED IN FIVE SEPARATE EXPERIMENTS AND EACH FIGURE IS MEAN DERIVED FROM FIFTY PLANTS

LEACHED NUTRIENT NO.	GROWTH (MM./10 PLANTS/10 DAYS) IN:				
	Leached nutrient		Leached nutrient diluted with fresh nutrient		Control (fresh nutrient)
	Millimeters	Percentage control	Three times	Nine times	
1.....	12.6	33	22.2	24.0	37.8
2.....	23.3	50	42.0	41.8	47.0
3.....	23.3	59	34.2	36.0	39.6
4.....	18.7	71	24.6	20.6	26.2
5.....	17.8	65	25.4	23.0	27.4
6.....	18.2	66	26.4	24.8	27.4
Average..	19.0 ± 1.63*	57.3 ± 5.68	29.1 ± 3.06	28.4 ± 3.46	34.2 ± 3.48

* Difference between this value and that of control significant at 1% level.

acidified material was much less effective in removing the toxic activity. These facts, which indicate that the toxic principle or principles may be acidic in nature, are brought out by data of typical experiments presented in table 8. Accordingly, attempts were made to enrich the toxic activity through a series of fractionations, and the result of a typical procedure is shown in table 9. By exhaustive extraction of the acidified dried recirculate with ether, most of the toxicity could be removed. The ether extract was then taken to dryness and the residue extracted with 2% NaOH. The ac-

city of material made it impossible to carry the fractionation substantially further than indicated in the table. From fraction 4b of table 9, 84 mg. of an unknown compound were isolated in crystalline form, m.p. 159°-161.5° C. Since this compound proved to be nontoxic, it was not investigated further. Fraction 4b also contained inactive metallo-organic compounds in considerable quantity.

About 1.8 gm. of ether-soluble material could be extracted from nutrient solution which had been recirculated over 240 guayule plants for 30 days

TABLE 8

EFFECT OF TREATMENTS ON TOXICITY TOWARD GUAYULE SEEDLINGS OF NUTRIENT RECIRCULATED OVER GUAYULE ROOTS. EACH FIGURE MEAN FROM TEN PLANTS. ALL FIGURES EXPRESSED IN PERCENTAGE OF CONTROL GROWN IN FRESH NUTRIENT. EACH FRACTION TESTED IN ALIQUOT EQUAL TO ORIGINAL FOR THAT EXPERIMENT

FRACTION	GROWTH IN HEIGHT (FULL STRENGTH)	PERCENTAGE CONTROL	
		Diluted three times	Diluted nine times
A: 1. Original recirculate.....	30%*	35	75
2. 1 concentrated down and again diluted.....	34*	72	77
3. Distillate from concentration.....	96	96	112
B: 1. Original recirculate.....	51*	66	74
2. 1 after ashing.....	98	104	102
C: 1. Original recirculate.....	51*	53	72
2. 96% EtOH extract.....	58*	69	98
3. Acid-ether extract.....	40*	63	85
4. Residue after 3.....	94	87	98
5. Neutral-ether extract.....	78	96	83
6. Petroleum-ether extract.....	110	106	88

* Fraction considered active.

TABLE 9

PROCEDURE IN FRACTIONATION OF TOXIC ACTIVITY FROM NUTRIENT SOLUTION PREVIOUSLY RECIRCULATED FOR 30 DAYS OVER GUAYULE CULTURES

Fraction	Dry weight (gm.)	Activity
1. 60 gallons recirculate, concentrated to dryness		
a. Dry residue.....	60	Active
b. Distillate.....		Inactive
2. 1a mixed with asbestos, acidified to pH 2.0, and extracted with peroxide-free ether		
a. Ether-soluble.....	1.81	Active
b. Ether-insoluble.....	58	Inactive
3. 2a reduced to small volume and extracted with successive portions of 2% NaOH		
a. Alkali-soluble.....	1.64	Active
b. Alkali-insoluble.....	0.16	Inactive
4. 3a acidified to pH 2.0 and precipitate filtered off		
a. Precipitated by acid.....	0.08	Very active
b. Not precipitated by acid (contains inorganic salts).....	1.49	Active

(table 9). This amounts to approximately 7.5 mg. per plant. In one experiment the volatile material in the recirculate was caught in a CO₂ trap during the concentration, and the distillate was exhaustively extracted with ether. This yielded a total of 0.7 gm. of volatile oils, or about 3 mg. per plant. Apparently, considerable quantities of organic matter accumulate in nutrient solution which is recirculated through guayule cultures. This organic matter includes, as just

TABLE 10

INHIBITION OF GROWTH OF GUAYULE SEEDLINGS BY ADDITION OF MATERIAL OBTAINED FROM WATER IN WHICH GUAYULE ROOTS WERE ALLOWED TO SOAK FOR VARIOUS PERIODS

EXP. NO.	DAYS SOAKED	HIGHEST CONCENTRATION OF DRIED MATERIAL (MG./CC.)	GROWTH IN HEIGHT (% CONTROL)		
			Highest concentration	Diluted three times	Diluted nine times
1.....	1	1.6	55	59	74
2.....	3	1.4	57	79	98
3.....	5	2.1	63	75	100
4.....	6	1.0	41	100	73
5.....	11	1.0	53	62	80

shown, fractions inhibitory to the growth of guayule seedlings.

STEPPING OF ROOTS.—Since isolation of the active compound from guayule recirculate was impeded by the small amounts of original material that could be obtained, even by relatively vast amounts of labor, various other methods of obtaining larger amounts were investigated. Of these the following was most successful. Roots of guayule nursery plants (0.25–0.50 inches, caliper at crown) were packed into 2-gallon jars and the jars filled with distilled water. After 1–11 days the water was drained off, concentrated, and the residue tested

for toxic activity on guayule seedlings. This material proved to be highly toxic (table 10), concentrations of 1–2 mg. per cubic centimeter giving roughly 50% inhibition of seedling growth. Steeping of the roots for more than 1 day did not appear greatly to increase the toxic activity of the solution. Solutions allowed to steep for as long as 11 days were still clear and relatively free of bacteria and possessed toxic activity toward guayule seedlings. The plants subjected to steeping also appeared healthy and could be readily transplanted after 11 days of treatment.

ISOLATION OF TOXIC COMPOUNDS FROM STEEPATE.—Table 11 gives the steps used in developing large quantities of material from steeping guayule roots. The weights and activities presented are those from two duplicate isolations. In each case approximately 20,000 nursery plants were used. These were topped at the crown, rinsed thoroughly in water, and packed tightly in a vertical position into 2-gallon glazed crocks, about 500 plants per crock. The roots were then covered with distilled water. After 4–6 days the water was drained off and the 65 gallons of light brown liquor filtered through cheesecloth, after which it was concentrated to dryness *in vacuo*. The dried material was acidified to pH 4.0 with H₂SO₄, transferred to a Soxhlet thimble, and extracted with peroxide-free ether for 32 hours. At the end of this time the mass was again acidified to pH 2.0 and extracted for an additional 24 hours. The ether extract was more toxic toward guayule seedlings than the original material, while the ether-insoluble residue was less toxic. The combined ether extracts were next concentrated to a small volume and extracted with ten to thirteen successive 20-cc. portions of 0.5 normal NaOH. This step did not

result in any marked enrichment of the activity but freed the ether extract of materials which would interfere with The acid-soluble fraction 4a was adjusted to pH 4 and after 2 days at 2° C. yielded 0.23 gm. of a crystalline material (5a).

TABLE 11
ISOLATION OF TOXIC COMPOUNDS FROM GUAYULE-ROOT STEEPATE.
ISOLATION NOS. 14 AND 15

Fraction	Weight (gm.)	Concentration for 50% inhibition (mg./cc.)
1. Dried material given off to distilled water by 20,000 guayule roots.....	460	1.5
2. Extraction of 1 (above) with redistilled ether		
a. Ether-soluble.....	16.9	0.5
b. Ether-insoluble.....	443	>3
3. Ether-soluble fraction 2a extracted with 0.5 normal NaOH		
a. Alkali-soluble.....	16.4	0.5
b. Alkali-insoluble.....	0.5	0.5
4. Fraction 3a acidified to pH 1.2		
a. Acid-soluble.....	16.0	0.4
b. Acid-insoluble (from isolation no. 15).....	0.72	0.036
5. Fraction 4a allowed to remain at 0° in water at pH 4.0 for 2 days		
a. Precipitate (crystalline).....	0.23	0.15
b. Filtrate.....	15.8	0.5
6. Fraction 4a distilled at atm. pressure		
a. Boiling at 63°-80° C.....	0.90	>2
b. Boiling at 80-99.....	0.40	>2
c. Boiling at 100-109.....	1.03	>2
d. Boiling at 110-119.....	1.46	>2
e. Boiling at 120-135.....	1.66	0.5
f. Boiling at 136-150.....	2.07	0.5
g. Residue.....	2.38	0.5
7. Fraction 6g taken up in water and extracted with benzene		
a. Benzene-soluble (partly crystalline) (from no. 15).....	1.51	0.1
b. Benzene-insoluble (from no. 15).....	0.47	>3
8. Fraction 4b subjected to sublimation in vacuo at 100° C.		
a. Sublimate (crystalline).....	0.50	0.036
b. Residue.....	0.22	>2

the next procedure. The alkali-soluble material was now acidified to pH 1.2 with concentrated H₂SO₄. After 5 days at 2° C. a copious crystalline precipitate appeared which was centrifuged off. This material (4b) was purified by sublimation, resublimation, three recrystallizations from petroleum ether, and four recrystallizations from water:

M.p.	Equivalent wt.	%C ²	%H
84°-86° C.	174	71.7	7.3

² Microanalyses by Dr. G. OPPENHEIMER and Mr. G. SWINEHART.

This was recrystallized three times from methanol and proved to be succinic acid:

	M.p.	Equivalent wt.	%C ²	%H
Found....	184.5° C.	60	40.8	5.40
Expected for succinic acid....	185.0	59	40.7	5.08

Fraction 5b was next distilled at atmospheric pressure and yielded a series of fractions boiling between 60° and 150° C. Of these only the two highest boiling

fractions possessed any marked toxic activity. The residue from the distillation was then taken up in water and extracted with successive portions of benzene. The benzene extract on evaporation yielded a crystalline material which proved to be trans cinnamic acid:

	M.p.	% C ^a	% H
Found.....	131°-133° C.	73.7	5.50
Expected for cinnamic acid.....	133°	73.0	5.40

It is to be noted that the total amount of activity present in the original steepate was not recovered in the acid-ether extract, or in this extract plus the residue. It may be that some loss of total activity accompanies this step. No procedure has as yet been devised which avoids this initial loss of activity. The fact that both of the active compounds obtained may be readily sublimed *in vacuo* at room temperature may account for this and other losses of activity.

Two highly active toxic compounds have been isolated from guayule root steepate—cinnamic acid and an unknown compound (4b). Since it has not been possible to isolate these compounds quantitatively, it cannot be stated with assurance how great a proportion of the total toxic activity of the acid-ether extract of the steepate is due to either. It would seem, however, that half or more of the toxic activity of the acid ether may be attributable to the compound 4b. Succinic acid, although somewhat toxic to guayule seedlings, accounts for only a negligible portion of the total toxic activity. Cinnamic acid is known, both in the free form and as the ester, from a wide range of plants, principally those containing essential oils or resins, as *Liquidambar* sp., *Styrax*, and *Cinnamomum* sp. The presence of cinnamic acid

in the free form in the guayule plant has been reported by WALTER (19).

ACTIVITIES OF PURE COMPOUNDS.—As a result of the identification of cinnamic and succinic acids in guayule-root steepate, these substances—and related ones—were tested for toxicity to guayule seedlings (table 12). Cinnamic acid would appear to be a potent inhibitor of guayule seedling growth, one-half inhibition being given by 30 mg. per liter of the pure material under the test condi-

TABLE 12

ACTIVITY OF PURE COMPOUNDS ON
GROWTH OF GUAYULE SEEDLINGS

Substance	Concentration (mg./l.) needed for 50% inhibition in height growth
1. Trans cinnamic acid.....	30
2. Cis cinnamic acid.....	30
3. Dihydrocinnamic acid.....	220
4. Phenylacetic acid.....	130
5. Parthenylcinnamate.....	Saturated solution inactive
6. Partheniol.....	Saturated solution inactive
7. Melilotic acid.....	45
8. O-coumaric acid.....	45
9. Coumarin.....	350
10. Succinic acid.....	120
11. Fumaric acid.....	200
12. Malic acid.....	120

tions. Figure 1 shows the relation between concentration and inhibition by cinnamic acid in the standard test. Cis cinnamic acid appeared to be at least as active as the trans isomer. It might be recalled that although cis cinnamic acid possesses auxin-like growth activity, the trans isomer is without such activity (5). Dihydrocinnamic acid (phenylpropionic acid) was five to ten times less toxic than trans cinnamic acid at the 50% inhibition level. It has been shown by WALTER (19) that a portion of the cinnamic acid in the guayule plant is present in the form of an ester with the sesquiterpene alcohol, partheniol. Parthenylcinnamate, a major constituent of guayule resin, is present in leaves, stems, and

roots and is exuded from the roots of guayule plants. Both partheniol and parthenylcinnamate were nontoxic toward guayule seedlings.

The hydroxy derivative of cinnamic acid, o-coumaric acid, is active in inhibiting guayule seedling growth, and the hydroxy derivative of dihydrocinnamic, melilotic acid, is equally so. The lactone,

nutrient solution, and in addition were watered once daily, either with pure water or with a solution of cinnamic acid. During the first 4 weeks as little as 1 mg. of cinnamic acid per liter significantly retarded growth, while at the expiration of 8 weeks significant reductions in dry-weight accumulation resulted from application of cinnamic acid.

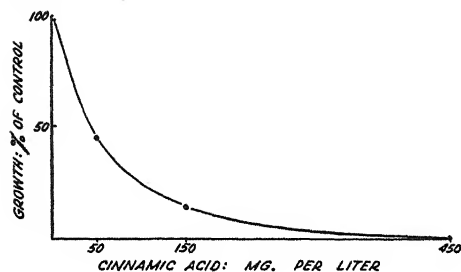


FIG. 1.—Influence of cinnamic acid on growth of guayule seedlings in unaerated solution culture. Growth given in percentage of that attained by control plants.

coumarin, of o-coumaric acid, however, is practically inactive. Fumaric and malic acids, both related to succinic, have—as the latter compound—only low inhibiting activity.

That cinnamic acid is inhibitory toward older guayule plants as well as toward seedlings is shown by the experiment of table 13. Nursery transplants, which had been topped nearly to the crown, were planted in 4-mesh gravel. The plants were supplied daily with

Discussion

It has been shown that certain organic compounds which arise from guayule roots and accumulate in the solution surrounding such roots are definitely toxic to guayule plants. These toxic agents, although they were not isolated in pure form, were characterized chemically as being ether-soluble acidic compounds. The toxic agents which were isolated in pure form from water, in which guayule roots had been briefly allowed to steep, fulfil these criteria. There can be, however, no certainty that the compounds isolated are in fact identical with those which arise from normally growing guayule plants. It should be borne in mind, however, that at least one of the toxic agents, cinnamic acid, is a normal constituent of the guayule plant.

The mode of liberation of the toxic substances which arise from guayule roots has purposely not been discussed in the present paper, since it is impossible at

TABLE 13
INFLUENCE OF CINNAMIC ACID ON GROWTH OF GUAYULE NURSERY
TRANSPLANTS; EXPERIMENT G-137

SOLUTION	HEIGHT (CM.) AFTER			FINAL DRY WEIGHT (GM./PLANT)	
	4 weeks	6 weeks	8 weeks	Stems	Leaves
Control (no cinnamic) ..	20.7±0.36	27.0±0.46	28.3±0.47	7.73	5.42
Cinnamic (1 mg./l.)	18.2±0.42	25.8±0.47	27.0±0.41	6.86	4.40
Cinnamic (10 mg./l.) ...	17.6±0.52	25.3±0.45	26.4±0.40	6.30	3.97
Cinnamic (100 mg./l.) ..	15.8±0.57	23.4±0.59	24.8±0.56	4.93	3.82

the present time to state whether they are liberated from dead or injured cells, excreted by living cells, or arise in some other way. That the intervention of bacteria is essential is a thesis made unlikely in view of the fact that cinnamic acid is a normal constituent of guayule.

The toxic compounds studied have been shown to inhibit growth of guayule under certain limited environmental conditions. Whether or not the same compounds are active in the soil under field conditions in inhibiting and limiting growth of guayule is a separate question which remains to be investigated.

Summary

1. The experiments indicate that substances unfavorable to the growth of guayule plants emanate from the roots of actively growing plants of this species. These substances are organic in nature and can be extracted from nutrient solu-

tion which has previously been flushed through gravel cultures of guayule plants.

2. The growth-inhibitory extracts were assayed for their toxic activity by application to guayule seedlings grown in solution cultures under standard conditions.

3. The growth-inhibitory substances contained in nutrient solution which has been recirculated through guayule cultures are ether-soluble acidic compounds. They were not, however, isolated in pure form.

4. A potent source of growth-inhibitory substance was found in water in which guayule roots were allowed to soak briefly. From this water two compounds inhibitory to the growth of guayule plants were isolated in crystalline form: cinnamic acid, and a second unidentified organic acid.

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EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE RIPENING OF DETACHED FRUIT

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Introduction

Late summer applications of aqueous sprays containing 2,4-dichlorophenoxyacetic acid to woody plants such as sumac, sassafras, and poison ivy resulted in marked changes in the color of the foliage of these plants. Sprayed leaves turned from green to red and yellow following treatment, in a manner similar to the coloration that occurs during the late fall. CHRISTOPHER (1) noted that attached fruit on apple trees which received preharvest "hormone" sprays developed a more intense color, and GERHARDT (2) observed that pears treated in a like manner ripened somewhat earlier than did unsprayed fruit. Furthermore, naphthaleneacetic acid and other growth-regulating substances have brought about an acceleration in the rate of starch hydrolysis when applied to the leaves of bean plants, a process which is associated with the ripening of certain kinds of fruit (8).

Experiments were therefore undertaken to determine the effects of growth-regulating substances on the rate of ripening of various kinds of fruit after removal from the plant. The effects of 2,4-dichlorophenoxyacetic acid on the ripening of apples, pears, bananas, tomatoes, peppers, and persimmons are reported here.

Methods

Fruits that differ widely in morphological and chemical constitution were used, namely: Grimes Golden, Yellow

Newtown, and Rome Beauty apples; Keiffer and Winter Bartlett pears; bananas designated in the trade by the varietal names of Fortuna and Guatemala; Marglobe tomatoes; World Beater and Paprika peppers; and Miller persimmons. These fruits all exhibit physical changes during ripening which are readily measured—such as changes in color, solidity, starch content, or taste.

The bananas were obtained from a local wholesale market soon after arrival. All the other fruits were selected for uniformity of size and color and collected from the growing plants immediately prior to treatment. After treatment, the various lots of fruits were kept in well-ventilated rooms at 65°–70° F. At the time of treatment and at following intervals, the color of individual fruits as it changed was measured by means of a Department of Agriculture Standard Ground Color Chart for Western Apples and Pears. The red color of the individual fruits was estimated as a percentage of their total surface area.

The firmness of the flesh was determined on samples selected at random periodically during the ripening period, as measured by means of a U.S. Department of Agriculture fruit pressure tester. This instrument measured in pounds the force required to insert a blunt plunger, $\frac{3}{8}$ inch in diameter, into the flesh of the fruit of both apples and pears or through the peel and flesh of bananas.

Methods employed in treating fruits with 2,4-dichlorophenoxyacetic acid consisted of dips and sprays of aqueous solutions and also the liquefied-gas aerosol method. Aqueous solutions were pre-

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pared by first dissolving the required amount of compound in Carbowax 400, which served as solvent, carrier, and spreader. The required amount of this wax-hormone mixture was then added to a measured amount of hot tap water. Carbowax 400 was used in the aqueous solutions in concentrations of 0.1%, 0.5%, 1.0%, and 2.0% (10). The following concentrations of 2,4-dichlorophenoxyacetic acid were applied in aqueous solutions with Carbowax: 100, 250, 500, 800, 1000, and 1600 p.p.m. Dip treatments were made by completely immersing the fruit for approximately 1 second. Finely dispersed aqueous sprays were applied by means of compressed-air sprayers of 1-quart capacity that operated under 60-90 pounds of air pressure. In some instances localized applications were made with a pipette or small brush. Ten fruits each were treated in the following ways: (a) 1 ml. of solution was placed in the stem cavity of each fruit, (b) 1 ml. was placed in the blossom end (basin) of each fruit, (c) a narrow band $\frac{1}{4}$ inch in width was "painted" around each fruit at its greatest circumference, and (d) the entire surface was treated by immersion in the solution.

The method used in preparing liquefied-gas aerosols of organic compounds has been described by MCGOVAN *et al.* (5). In treating by this method, an aerosol-forming mixture composed of 94% dimethyl ether, 5% cyclohexanone, and 1% 2,4-dichlorophenoxyacetic acid was made by weight. These mixtures were stored in cylinders of 1-pound capacity and were later sprayed on the fruits in the open air. Control lots were treated with a similar gas aerosol not containing the acid.

Statistical analysis of the data, both of the color readings and of the pressure tests on individual fruits, was by analysis of variance. Differences between treat-

ments that exceed the 5% level are referred to as significant.

Results

BANANAS

Stems of bananas were obtained on September 18 and on October 16 from storage rooms of wholesale dealers in Washington, D.C. The different lots of fruit had apparently received varied treatment with respect to conditions under which they had been grown and harvested, as well as under which they had been stored following harvest. Although the fruit on all stems was classified as " $\frac{3}{4}$ filled," they differed greatly in moisture content. The fruit obtained on September 18 was relatively fresh, as indicated by the fact that juice exuded readily from cuts made in the stem or fruit, while fruit obtained a month later was desiccated to the extent that juice did not flow from the cut surface when the stem was severed or the fruit freshly cut.

Fruit of the initial shipment, which was hard and deep green in color, was treated on September 18 as previously described. During the 24-hour interval immediately following treatment, those of the Fortuna lot to which 2,4-dichlorophenoxyacetic acid had been applied in concentrations of 200, 800, or 1600 p.p.m. changed from a deep green to a yellowish green color. Seventy-two hours after treatment, all the Fortuna fruits to which the acid solutions had been applied were completely yellow in color but firm to the touch, while the untreated fruits were hard and still deep green in color. Five days after treatment, fruits that received solutions containing 200, 800, or 1600 p.p.m. of the acid were ripe and of excellent flavor, while untreated fruits were still light green in color, bitter in flavor, and hard to the touch (table 1).

Iodine tests made on this date indicated that untreated fruits contained a much greater percentage of starch than did those to which solutions of the acid had been applied.

Ripening of the Guatemala variety was also hastened by application of 2,4-dichlorophenoxyacetic acid, but the time required for sprayed fruit to become entirely ripened was 1-2 days longer than for Fortuna. After all fruit had ripened, tests indicated that the quality of acid-treated fruit equaled, and in many cases exceeded, that of untreated fruit.

fruits were still hard and light green in color 9 days after treatment, while those to which the acid had been applied were completely yellow, or yellow with brown flecks, and fully ripe at the end of 5 days.

Relatively low concentrations (100 p.p.m.) were more effective in hastening ripening than were higher concentrations (1000 p.p.m.). Application of the acid by the aerosol method was slightly more effective than was the spray treatment.

Aqueous spray solutions that con-

TABLE 1
RELATIVE SOFTNESS OF FORTUNA BANANAS FOLLOWING SPRAY TREATMENT
WITH 2,4-DICHLOROPHENOXYACETIC ACID. TREATED SEPTEMBER 18;
PRESSURE READINGS 5 DAYS LATER

Concentration of acid (p.p.m.)	Averages of three readings per fruit (in pounds) Eight fruits per treatment								General average
	30*	30	30	30	30	30	30	30	
Control.	30*	30	30	30	30	30	30	30	30.00
200.	13.3	12.0	13.6	14.0	11.0	14.0	16.6	12.3	13.37
800.	11.3	12.6	12.0	14.6	12.3	13.0	16.0	12.3	13.33
1600.	15.3	12.0	13.3	16.3	14.0	13.6	12.6	13.3	13.81

* Or over 30, the maximum for the tester used.

Fortuna bananas obtained on October 16 exhibited marked response to treatment with the acid, but ripening extended over a relatively long period of time, possibly owing to the fact that the fruit had been longer in transit than those of the previous experiment, or because they had been grown and harvested under a different environment. However, applications of solutions containing concentrations of 100 or 1000 p.p.m. greatly accelerated ripening (table 2). As in the earlier experiments, color change from green to yellow was associated with a decrease in hardness of the fruit and an improvement in its flavor, as indicated by taste tests made as the fruit ripened. Under the conditions of this experiment, the untreated

tained Carbowax 400 alone in concentrations of from 0.5 to 2.0% did not hasten the ripening of bananas. Solutions that contained 2,4-dichlorophenoxyacetic acid and 2% Carbowax were no more effective in hastening ripening than were others that contained only 0.5% of Carbowax and an equal amount of the acid. A weak alcoholic solution containing the acid was less effective in hastening ripening than was an aqueous solution of Carbowax that contained an equal amount of the acid (table 2).

APPLES

GRIMES GOLDEN.—Fruits that were still green in color were collected on September 12 and dipped in aqueous solutions containing 500 or 1000 p.p.m.

of the acid. They were almost fully yellow 6 days later. At the end of 11 days those treated with a lower concentra-

Seventeen days after treatment, fruits in all treated lots were completely yellow in color, but fleshy parts of treated fruits

TABLE 2

RELATIVE RATE OF COLORATION OF GREEN FORTUNA BANANAS FOLLOWING TREATMENT WITH 2,4-DICHLOROPHENOXYACETIC ACID. TREATMENTS APPLIED AS AQUEOUS SPRAYS AND BY AEROSOL METHOD, ON OCTOBER 16

TREATMENT	No. OF FRUITS	AVERAGE COLOR READING				
		Oct. 19	Oct. 21	Oct. 23	Oct. 24	Oct. 25
Untreated control.....	41	1.00	1.80	2.10	2.50	2.75
2.0% Carbowax sprayed control..	21	1.00	1.10	1.66	2.66	2.80
2.0% Carbowax; 100 p.p.m. of acid; sprayed.....	21	2.50	3.40	3.75	4.00	4.00
0.0%* Carbowax; 100 p.p.m. of acid; sprayed.....	21	1.00	1.75	2.22	2.80	3.00
2.0% Carbowax; 1000 p.p.m. of acid; sprayed.....	20	1.00	2.80	3.50	3.90	4.00
0.5% Carbowax; 1000 p.p.m. of acid; sprayed.....	23	1.00	2.66	3.40	3.90	4.00
2, 4-dichlorophenoxyacetic acid; aerosol.....	20	3.00	4.00	4.00	4.00	4.00

* Acid first dissolved in 0.5 ml. of 95% ethyl alcohol.

TABLE 3

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID APPLIED IN AQUEOUS SOLUTION AS DIPS AND BY LIQUEFIED-GAS AEROSOL METHOD ON RELATIVE RATE OF CHANGE FROM GREEN TO YELLOW OF GRIMES GOLDEN APPLES. TREATED SEPTEMBER 12

TREATMENT	AVERAGE COLOR RATING OF TEN FRUITS				PRESSURE TEST IN POUNDS AV. OF TEN FRUITS AND SUM OF THREE READINGS PER FRUIT
	Sept. 18	Sept. 21	Sept. 25	Sept. 29	
Untreated control.....	1.5	2.3	2.2	2.6	42.0
Dipped control*.....	1.8	2.2	2.2	2.9	38.0
Dipped 250 p.p.m.....	2.4	3.5	3.8	4.0	31.0†
Dipped 500 p.p.m.....	3.1	3.5	3.8	4.0	29.5†
Dipped 1000 p.p.m.....	3.6	3.6	3.9	4.0	30.6†
Aerosol without acid.....	1.8	2.1	1.9	2.8	41.0
Aerosol with 1% acid.....	2.1	2.7	3.4	4.0	32.3†

* All aqueous dip treatments contained 0.5% of Carbowax 400.

† Statistically significant difference from untreated controls at 5% level (difference of 8.64 required).

tion (250 p.p.m.) likewise had turned yellow (table 3). Comparable untreated fruit failed to color during this 11-day period, as indicated by color readings.

were significantly softer in texture than those of untreated, as indicated by pressure tests.

In another experiment, freshly picked

Grimes Golden apples were dipped in solutions containing 100 and 1000 p.p.m. of the acid on September 19. The fruits treated with 1000 p.p.m. were fully yellow 6 days after treatment, and were

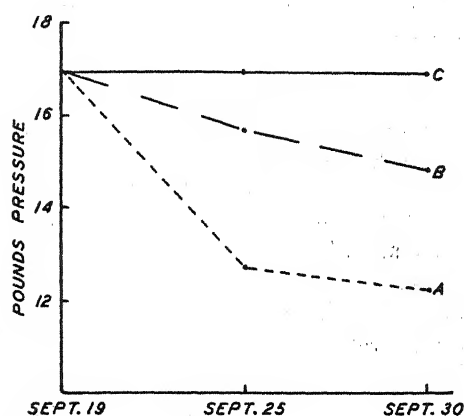


FIG. 1.—Rate of softening of Grimes Golden apples dipped: A, in solution containing 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid; B, in solution containing 100 p.p.m.; C, untreated fruits. Average pressure-test readings of ten fruits from respective treatments at each sampling date.

sweet in flavor and mellow (low pressure-test reading). Application of 100 p.p.m. of the acid likewise resulted in a statistically significant reduction in pressure-test readings as compared with those of the untreated fruits, at the end of both 6 days and 11 days following treatment (fig. 1). Final pressure readings obtained 17 days after treatment showed no significant difference between any of the treated or control lots. On this date, however, the controls were still relatively green in color (table 4) but had softened to about the same degree as the fruits in both treated lots.

It has been demonstrated repeatedly that the effects of application of growth-regulating substances to plants are often manifest at some distance from the point of application. An experiment was made to determine the effects of localized ap-

plication of 2,4-dichlorophenoxyacetic acid on the rate of ripening of Grimes Golden apples. At the end of 11 days following treatment it was apparent from the standpoint of color that the ripening effect of treatment with the acid was localized. Treatment of the entire fruit, either by the dipping or the aerosol method, resulted in complete yellowing within 6-8 days following treatment. The fruits treated in localized areas were still green in color (rating 2 on color chart), except in those areas to which the acid solution was applied. These treated areas became yellow (rating 4 on color chart) and clearly defined within a period of 6-8 days following treatment. There was a suggestion, however, that treatments applied on the stem cavity had brought about a more general color change than had applications made on the blossom end (calyx)

TABLE 4

RELATIVE RATE OF COLOR CHANGE OF GRIMES GOLDEN APPLES FOLLOWING DIP TREATMENT IN AQUEOUS SOLUTIONS OF 2,4-DICHLOROPHENOXYACETIC ACID. TREATED SEPTEMBER 19

TREATMENT	AVERAGE COLOR READING PER TREATMENT				
	128 fruits	32 fruits	24 fruits	16 fruits	8 fruits
	Sept. 19	Sept. 22	Sept. 25	Sept. 30	Oct. 4
Untreated.....	1.7	1.9	1.6	2.3	2.9
Dipped control*....	1.7	1.8	2.2	2.6
Dipped 100 p.p.m..	1.8	2.0	3.0	3.1
Dipped 1000 p.p.m.	1.7	2.2	3.6	4.0

*All dip solutions contained 0.5% Carbowax 400.

or around the greatest perimeter of the fruit.

YELLOW NEWTOWN.—The effect of 2, 4-dichlorophenoxyacetic acid on the ripening of this variety of apple was de-

terminated in order to compare the response of a late-fall variety with that of an early-ripening variety, such as Grimes Golden. Dip treatments at both 100 and 1000 p.p.m. concentrations applied on October 5 had no significant effect on pressure-test readings of the fruit at the end of an 18-day period following treatment. During the first 11 days following treatment, however, many of those treated with 1000 p.p.m. had developed a full yellow color, and at the end of 18 days practically all those treated with the 100 or the 1000 p.p.m. concentration were rated as full yellow in color (4 on color chart). In contrast, untreated fruits kept under the same conditions were still light green in color on the eighteenth day following treatment. Eighteen days following treatment the untreated fruits were sour and "starchy" in taste, while those treated with either 100 or 1000 p.p.m. were sweet and of good flavor.

ROME BEAUTY.—Fruits in the "mature-green" stage of maturity were carefully selected for uniformity of both red and green ground color. In general, the rate of softening and the changes in green ground color followed closely the results obtained with Grimes Golden. Twelve days following treatment, those fruits to which solutions containing 100 and 1000 p.p.m. had been applied were significantly softer than were untreated fruits. At the end of an 18-day period following treatment, the fruits in both treated and control lots were relatively soft (10–12 lb. pressure test). Color readings showed that the acid markedly reduced the intensity of the green ground color at both sampling days (11 and 18 days after treatment), and that the intensity of yellow color increased, thus greatly improving the appearance of the fruit. Eighteen days following treatment

most of the untreated fruits were green and red, while the majority of treated fruits were yellow and red in color. Estimations showed that there was no appreciable difference in the percentage of red surface area on treated and untreated fruits.

PEARS

WINTER BARTLETT AND KIEFFER.—Although both of these are late-season varieties, they differ considerably in the manner in which the fruits ripen when harvested. Those of the Kieffer variety are usually harvested 7–10 days earlier, but the fruits generally remain firm for a relatively long period of time after being picked. Winter Bartlett pears usually soften very quickly under comparable handling conditions, even though the fruits may be much harder than Kieffer at the time of harvest.

Dip treatments, using aqueous solutions containing either 100 or 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid, hastened the rate of softening of fruits of both varieties, and treatment at 1000 p.p.m. on September 20 hastened the rate of softening of Kieffer pears so that there was no appreciable difference in the firmness of fruits of the two varieties when measured 2 weeks later (table 5). The rate of ripening of individual treated fruits varied less than did that of untreated fruits, as indicated by pressure and color tests made at the end of the experiment.

Winter Bartlett pears were so russeted that reliable color readings could not be obtained. Readings were obtained on Kieffer, however, at periodic intervals following treatment. Those treated with the 1000 p.p.m. concentration were almost fully yellow (3.3. on color chart) on the eighth day following treatment, while controls at this time were green in

TABLE 5

EFFECT OF AQUEOUS DIP TREATMENTS OF 2,4-DICHLOROPHENOXYACETIC ACID ON RELATIVE RATE OF SOFTENING OF WINTER BARTLETT AND KIEFFER PEARS AS INDICATED BY PRESSURE TEST. TREATED SEPTEMBER 26

CONCENTRATION OF ACID (P.P.M.)	PRESSURE TEST IN POUNDS, AVERAGE OF TEN FRUITS					
	Oct. 3		Oct. 6		Oct. 10	
	Winter Bartlett	Kieffer	Winter Bartlett	Kieffer	Winter Bartlett	Kieffer
0 (Dry).....	25.3	23.6	24.1	23.2	14.5	17.7
0 (Dipped)*.....	26.5	23.1	27.7	23.8	13.2	17.7
100 (Dipped).....	26.5	21.0	15.4	18.6†	5.6†	9.6†
1000 (Dipped).....	23.2	19.2	18.7	20.2	5.0†	5.1†

* Carbowax 400 used in all dipped lots at 0.5% concentration.

† Significant effect of treatment at 5% level (difference required: Winter Bartlett 7.18, Kieffer 4.66).

color (2.1 and 2.2 on color chart). At the end of 10 days following treatment, the treated fruits in both lots (100 p.p.m. and 1000 p.p.m.) were fully yellow (table 6). During the experiment, individual

TABLE 6

EFFECT OF AQUEOUS DIP TREATMENT OF 2,4-DICHLOROPHENOXYACETIC ACID ON RELATIVE RATE OF COLOR CHANGE BY KIEFFER PEARS. TREATED SEPTEMBER 26

CONCENTRATION OF ACID (P.P.M.)	AVERAGE COLOR READING OF FIFTY FRUITS			
	Oct. 3	Oct. 4	Oct. 6	Oct. 9
Untreated control.....	1.9	2.1	3.0	3.5
0*.....	2.2	2.2	3.0	3.8
100.....	2.6	2.6	4.0	4.0
1000.....	3.2	3.3	4.0	4.0

* Carbowax 400 used in all dipped lots at 0.5% concentration.

fruits of treated lots developed yellow color uniformly, while individual fruits of untreated lots varied widely in the rate at which they changed from green to yellow. This response was perhaps the most significant of any noted in connection with chemically controlled ripening of pears.

MARGLOBE TOMATO, WORLD BEATER PEPPER, PAPRIKA PEPPER, AND MILLER PERSIMMON.—Treatment with 2,4-dichlorophenoxyacetic acid, either as aqueous-solution dips or by the liquefied-gas aerosol method, failed to affect the rate of coloring or softening of any of these fruits. In all instances from ten to twenty mature green fruits were treated in the same manner and held under the same conditions that resulted in marked color changes and softening of such fruits as bananas, apples, and pears.

Discussion

The ripening of pears (2) treated with growth-regulating substances, such as naphthaleneacetic acid, to prevent pre-harvest drop is sometimes hastened; and apples, treated in a similar manner, may attain a more brilliant color than do other unsprayed ones on the same tree (1). Tomato fruits are known to respond in a similar way when treated with growth-regulators while yet attached to the vine (4). These effects may result from an increase in the rate at which materials are mobilized from other parts of the plant into the fruit (6), from the

activation or acceleration of chemical and physical processes associated with ripening (9), and possibly from an increase in the over-all vigor of the treated portion of the plant (7). In contrast, acceleration of the ripening processes of detached fruits must necessarily be associated with the activation or acceleration of chemical and physical changes which occur within the fruit. One such process, which is common to most starchy fruits during the ripening period, is the gradual conversion of starch to sugars. Based on results presented, diastatic activity in fruits such as apple, banana, and pear can be accelerated by the application of 2,4-dichlorophenoxyacetic acid. Fruits such as peppers, tomatoes, and persimmons, which are generally without an appreciable starchy reserve, failed to respond to treatment with this acid, as measured by their rate of color change during ripening. It remains to be learned whether the ripening processes of nonstarchy fruits, other than those concerned with external coloration, can be influenced by treatment with growth-regulating substance.

HALLER (3) has reported that the softening of apples during ripening is associated with the conversion of insoluble forms of pectin to soluble forms, thereby making the cell wall less resistant to pressure. Treatment with 2,4-dichlorophenoxyacetic acid may likewise have accelerated the rate at which this conversion process occurred in the pears and apples, thus accounting for the rapid decrease noted in the resistance of the tissues to pressure.

Through the use of growth-regulating substances such as this acid,³ to-

³ 2,4-Dichlorophenoxyacetic acid is relatively nontoxic, since 200 mg. of the acid was fed daily to small experimental mammals with no apparent ill effects.

gether with regulation of temperature, it may be possible to control to a greater extent the rate of ripening of fruits that have a starchy carbohydrate reserve by accelerating their rate of starch hydrolysis and color formation at the desired time. For instance, it is sometimes desirable to ripen fruits as soon as possible after harvesting, or after removal from storage. Based on responses observed in the present experiments, the use of 2,4-dichlorophenoxyacetic acid might also be of benefit in bringing about a more uniform rate of ripening among individual fruits, such as those of pears, which often ripen unevenly after harvest.

Summary

1. 2,4-Dichlorophenoxyacetic acid was applied to the detached fruits of bananas, apples, pears, tomatoes, peppers, and persimmons and its effect on the rate of ripening of these fruits observed. The fruits were collected while still unripe and sprayed with or dipped in aqueous solutions of the acid, which contained 0.5%–2.0% Carbowax. Other fruits comparable in stage of development were treated with the acid by the liquefied-gas aerosol method, and all were then stored at room temperature.

2. Based on color changes, the ripening period of bananas was shortened from 3 to 8 days following treatment. Treated fruits attained a full yellow color and excellent flavor, even though they were subjected to drier air and lower temperature conditions than is usually maintained in their commercial ripening. Yellow Newtown apples ripened during the 2 weeks' period immediately following treatment, whereas comparable untreated apples failed to ripen in this length of time. Grimes Golden apples treated with the acid approximately 1 week before the usual picking date, when

still green in color, ripened within a period of 6 days immediately following treatment. Untreated fruits picked at the same time required approximately 2 weeks at room temperature to reach the same stage of ripeness. Rome Beauty apples treated with the acid ripened 2-6 days earlier than the untreated ones, as indicated by both pressure tests and color readings.

3. The time required for Kieffer pears to change from green to yellow, when collected and treated approximately 10 days before the usual picking date, was 2-4 days less than required for comparable untreated fruits. Winter Bartlett pears ripened within a period of 8-10

days following treatment, while untreated fruits failed to ripen during this period. Treated fruits of both varieties of pears ripened more uniformly than the controls.

4. Based on color changes, the rate of ripening of fruits of tomato, pepper, and persimmon was not affected by treatment with the acid.

5. Starch hydrolysis proceeded at a more rapid rate during the ripening of treated bananas than it did during the ripening of untreated fruits, as indicated by both iodine and taste tests.

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SOME ANATOMICAL EFFECTS OF MOISTURE STRESS IN NURSERY SEEDLINGS OF GUAYULE

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Introduction

The effects of moisture on the development of guayule seedlings (*Parthenium argentatum* Gray) and their behavior subsequent to transplanting have been the subject of considerable study by the Soils Division of the Special Guayule Research Project. As part of this investigation, a limited study of the anatomical effects of moisture stress in nursery seedlings was undertaken.

MATERIAL AND METHODS.—Material was secured December 13, 1943, from experiments being conducted (5) at the Alisal Nursery, Salinas, California. A sample of ten plants was taken from each of three treatments: I, low moisture stress; III, intermediate moisture stress; V, high moisture stress.

The beds were seeded June 3, 1943, and all plants were given the same amounts of moisture until July 13, at which time the differential treatments were applied. Prior to seeding, the soil of all plots was wet to field capacity to a depth of at least 6 feet. In treatment I the moisture was kept between field capacity and a tension of 450 cm. of water at a depth of 6 inches until September 14. In treatment III it was attempted to keep the moisture between field capacity and a tension of 850 cm. at the 6-inch depth until August 30. Treatment V received no irrigation after July 13. The tension at lower depths in treatments I and III never exceeded that at the 6-inch depth. Rainfall between the end of moisture applications

and the time of collection of samples was negligible.

Sections were made from (a) the root-crown, (b) a young stem, and (c) mature leaves of the plants in each sample. The root-crown was considered to be that portion of the axis derived from the hypocotyl; therefore this zone was the same age in all treatments. Sections from this region should be comparable, except for the pith, inner xylem, and primary phloem fibers; it was not possible to obtain sections showing similar degrees of transition in these tissues. Sections of young stem were taken from the internode showing the greatest elongation. It is uncertain whether these were comparable as to age, but since they had developed during the period in each treatment most favorable for growth in length, it was considered permissible to compare observations of the pith and other primary tissues which had matured immediately following elongation. Leaf sections were made from a band of tissue cut across the central portion of the leaf about 5 mm. wide. The leaves were those of recent development. They had all appeared after the differential moisture treatments had been terminated; hence it might not be anticipated that they would show many differences.

The material was fixed in formalin-propiono-ethanol and sectioned at 70 μ on a sliding microtome using a freezing attachment. The sections were stained with the oil blue-safranin-Congo red combination recently described (1). This staining showed the distribution of rubber and defined the principal tissues. Observations of stem, root-crown, and

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certain of those of leaves were made from sections stained with this combination. In addition, a portion of each leaf sample was imbedded in paraffin, sectioned, and stained with safranin-haematoxylin (4). Leaf measurements were made from these sections, as this stain permitted more detailed examination of leaf structure.

Measurements were made of four sections from each block of tissue, using an ocular micrometer. Calculations of areas were based on the following linear measurements:

Radius: from center of pith to outer edge of cork.

Pith: from center of pith to inner edge of xylem.

Xylem: from outer edge of pith to cambium.

Cortex-phloem: from cambium to phellogen.

(For convenience and accuracy these two tissues were combined.)

In the root-crown, where the pith is in transition, the xylem measurements were taken from the center of the section to the cambium. The figures given for areas of xylem in the root-crown therefore include varying amounts of pith. The measurements were converted into percentage of area, and analyses of variance were made. Measurements of gross external characteristics—as well as the results of chemical analyses on the plants of these experiments—are being published by KELLEY *et al.* (5).

Observations

The results of measurements are presented in tables 1-3. Table 1 summarizes the data from cross-sections of the root-crown. In general, the observations are as would be expected. The area of the sections increases with increasing availability of water. However, the development of each of the major tissues does not parallel the amount of water available to the plant. For example, the cork

remains the same in each treatment with respect to both the total thickness of the layers and the width of the individual cells. The xylem and cortex-phloem show some interesting relationships. Whereas the thickness of the xylem increases directly with increasing availability of water, the development of the cortex-phloem tissues does not parallel that of the xylem. There is no significant difference between the cortex-phloem tissues in treatments III and V. Thus the increased amount of water available in treatment III over V is reflected in xylem development but not in cortex-phloem development. Under treatment I, which had the largest amount of available water, both xylem and cortex-phloem show significantly greater development than in the other two treatments. Apparently, under the conditions of treatment III, the development of xylem has priority over the development of cortex-phloem. ARTSCHWAGER (2) has made a similar observation relating to the tissues derived from the cambium as growth is resumed in the spring. During the first months of active growth he found that the development of xylem proceeds much more actively than the development of phloem.

For purposes of comparison, the proportionate areas of xylem, cortex-phloem, and cork were included in table 1. These figures, however, do not appear to be as illuminating as the absolute measurements.

At the level of the root-crown, the absolute width of the rays or the width in terms of number of cells is not affected by moisture stress.

The more narrow width of the cortical cells of plants grown under conditions of low moisture stress does not indicate that the cortex is small in this case. In view of the increased diameter of the

root-crown, the reduced width of the cortical cells appears to be caused by tangential stretching, and/or radial compression. Both of these were observed in the sections (figs. 1, 2). It may be pointed out in this connection that the cortex of guayule retains a low level of meristematic activity throughout the life of the

the maximum elongation. They are therefore not comparable in all respects. These internodes developed during the time the treatments were being applied, however, and should be comparable with respect to their primary tissues. Measurements of total area, xylem, cortex-phloem, and cork, each of which may

TABLE 1
MEASUREMENTS FROM CROSS-SECTIONS OF ROOT-CROWN OF PLANTS GROWN
UNDER THREE DEGREES OF MOISTURE STRESS

	MOISTURE STRESS			DIFFERENCE REQUIRED FOR SIGNIFICANCE*	
	I Low	III Intermediate	V High	0.05	0.01
Total area of section..	40.0 sq. mm.	21.1 sq. mm.	18.4 sq. mm.	5.2 sq. mm.	6.7 sq. mm.
Total radius of section.	3.59 mm.	2.77 mm.	2.42 mm.	0.30 mm.	0.41 mm.
Width of xylem (radius)†.....	2.19	1.67	1.22	0.14	0.18
Width of cortex-phloem†.....	1.10	0.78	0.87	0.14	0.18
Width of cork†.....	0.30	0.33	0.33	Not sig.	Not sig.
Percentage of area:					
Xylem.....	37.0%	36.0%	25.5%	3.9%	5.5%
Cortex-phloem.....	47.0	41.5	49.0	3.0	4.2
Cork.....	16.0	22.5	25.5	4.3	5.8
Total.....	100.0	100.0	100.0
Width of cork cells...	0.077 mm.	0.071 mm.	0.073 mm.	Not sig.	Not sig.
Width of cortical cells.	0.032 mm.	0.032 mm.	0.038 mm.	0.002 mm.	0.003 mm.
Width of vascular rays	0.049 mm.	0.048 mm.	0.048 mm.	Not sig.	Not sig.
No. of cells in ray....	2.97	2.9	2.8	Not sig.	Not sig.

* If treatments differ more than the figure given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the difference was not due to chance.

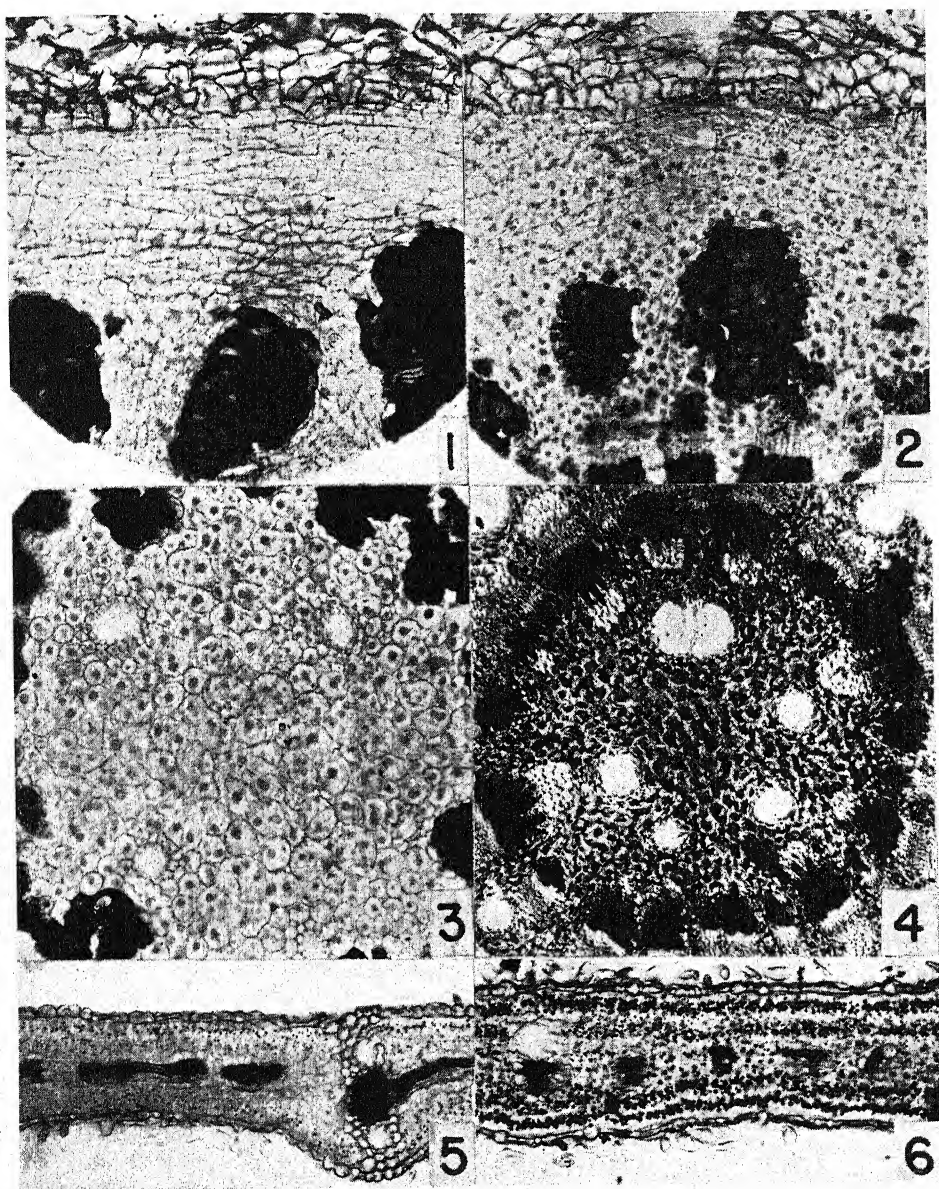
† An analysis of variance showed a highly significant interaction between tissues and treatments with a difference between differences of 0.19 required to give odds greater than 19:1 and 0.26 required to give odds greater than 99:1 against such differences being due to chance.

plant. As the stem enlarges, radial divisions in the cortex maintain the integrity of the tissue. It is therefore possible to identify the cells derived from the primary cortex, even in the root-crown region of 13-year-old plants. This phenomenon is also known in other woody plants (3).

In considering the data of table 2, it should be recalled that the material from which these sections were obtained was the internode of the plant which showed

consist entirely or largely of secondary tissue, probably are of little value in this instance. They are included to indicate the general state of development of the material studied.

Among all treatments there was no significant difference in length of the cortical or epidermal cells. While the diameter of the pith in treatment I is considerably larger than in the other treatments, the number of cells along this diameter is not significantly greater.



FIGS. 1-6.— Sections stained for rubber content, which appears as dark globules within parenchymatous cells. Figs. 1, 3, 5, from treatment I (low moisture stress). Figs. 2, 4, 6, from treatment V (high moisture stress). Figs. 1, 2, root-crown showing cork, cortex, and phloem. Figs. 3, 4, pith of young stem; note resin canals. Figs. 5, 6, leaves. Stem and root-crown sections are 70μ thick. $\times 80$.

The increased diameter is therefore directly correlated with the larger diameter of the pith cells in treatment I.

The most striking effect of moisture stress in primary tissues of young stems is on the resin canals (figs. 3, 4). Treat-

Several other effects were noted in the sections of the root-crown and of the young stem which could not be readily measured by the means at our disposal; nevertheless, they appeared consistently. As the available moisture was reduced,

TABLE 2
MEASUREMENTS FROM CROSS-SECTIONS OF YOUNG STEM OF PLANTS GROWN
UNDER THREE DEGREES OF MOISTURE STRESS

	MOISTURE STRESS			DIFFERENCE REQUIRED FOR SIGNIFICANCE*	
	I Low	III Intermediate	V High	0.05	0.01
Percentage of area:					
Xylem.....	24.8%	23.6%	16.1%	4.9%	7.2%
Cortex-phloem.....	48.4	54.4	52.5	Not sig.	Not sig.
Cork.....	14.0	9.6	15.6	3.8%	5.6%
Pith.....	12.8	12.4	15.6	Not sig.	Not sig.
Total.....	100.0	100.0	100.0		
Total area of section..	5.7 sq. mm.	3.1 sq. mm.	2.2 sq. mm.	0.8 sq. mm.	1.1 sq. mm.
Total radius of section..	1.337 mm.	0.994 mm.	0.836 mm.	0.103 mm.	0.138 mm.
Width of xylem (radius)	0.347	0.248	0.145	0.051	0.067
Width of cortex-phloem	0.411	0.343	0.299	0.035	0.047
Width of cork.....	0.096	0.048	0.069	0.010	0.013
Length of cortical cells.	0.043 mm.	0.042 mm.	0.045 mm.	Not sig.	Not sig.
Length of epidermal cells.....	0.073 mm.	0.063 mm.	0.068 mm.	Not sig.	Not sig.
Diameter of pith.....	0.937 mm.	0.690 mm.	0.660 mm.	0.092 mm.	0.123 mm.
No. of cells in diameter of pith.....	19.8	18.5	19.0	Not sig.	Not sig.
Av. diameter of cells in pith.....	0.047 mm.	0.037 mm.	0.035 mm.	0.005 mm.	0.006 mm.
Av. area of resin canals in pith.....	0.006 sq. mm.	0.012 sq. mm.	0.026 sq. mm.	0.012 sq. mm.	0.016 sq. mm.

* If treatments differ more than the figure given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the difference was not due to chance.

ment V, which received the least amount of moisture, had the largest resin canals. In this treatment the canals had more than four times the cross-sectional area of the canals in the plants that received the greatest amounts of moisture. This phenomenon appears to be related to the frequent observation that low amounts of available moisture lead to greater amounts of rubber and resins per unit of weight in guayule (7).

the amounts of rubber and lignin deposited increased. This effect was striking in the case of rubber, many of the sections from treatment V showing conspicuous amounts of stained rubber—even to the unaided eye. In the case of lignin deposition, the effects were less obvious but detectable in the more nearly complete lignification of the fibers in treatment V. This was less often the case as the available water was increased. As

would be expected, the increased deposition of rubber and lignin is closely correlated with the accumulation of carbohydrates within the plants (8).

Table 3 summarizes the measurements and counts from sections of the fully expanded mature leaves from the three treatments. In none of these did the leaves differ significantly. This is probably due to the fact that all treatments had gone without irrigation for 3 months prior to the time of sampling.

in the increased deposition of rubber within them.

It is apparently difficult to modify the fundamental pattern of development in guayule. LLOYD (6) noted but little difference in the structure of leaves from plants grown under both extremes of moisture. ARTSCHWAGER (2) found that "plants of a given variety show little difference in sclerenchyma development under varying soil moisture stresses." This is an important observation, since

TABLE 3
MEASUREMENTS FROM CROSS-SECTIONS OF LEAVES GROWN
UNDER THREE DEGREES OF MOISTURE STRESS

	MOISTURE STRESS			DIFFERENCE REQUIRED FOR SIGNIFICANCE	
	I Low	III Intermediate	V High	0.05	0.01
Leaf thickness.....	0.270 mm.	0.256 mm.	0.269 mm.	Not sig.	Not sig.
Length of parenchyma cells in midrib.....	0.170 mm.	0.181 mm.	0.172 mm.	Not sig.	Not sig.
Av. length of cells in mesophyll	0.045 mm.	0.042 mm.	0.042 mm.	Not sig.	Not sig.
No. of cells thick.....	8.0	8.1	8.4	Not sig.	Not sig.

Leaves which had developed under the differential moisture treatment had been lost, and the ones sampled had developed under closely similar moisture conditions in the soil. It is reasonable, therefore, to expect few differences in this material. It should be added, however, that the leaves from the three treatments showed great dissimilarity in the amount of rubber they contained. As in the stem sections, the lower the available moisture the more rubber was present (figs. 5, 6). It is possible that this was related to the amount of rubber in the plants of the different treatments. A relatively high concentration of rubber in the stem and root of a plant might lead to the accumulation of the precursors of rubber in the leaves and result

the sclerenchyma fibers of the cortex-phloem region take up space which would otherwise be occupied by rubber-bearing parenchyma. The data presented here support and extend these findings. The thickness of the cork is not affected by moisture stress, nor is the width of the vascular rays. Neither is the length of cells in the cortex and epidermis affected, nor the width of cells in the cork and vascular rays. It may be, therefore, that these anatomical structures are controlled by genetic factors not readily influenced by the physiological condition of the plant.

Moisture stress did affect the size of the pith and the tissues laid down by the cambium. Low moisture stress leads to a larger pith and greater cambial activi-

ty. High moisture stress leads to greater deposition of rubber and lignin, as well as to marked enlargement of the resin canals. Most of these observations would be expected from a knowledge of the effects of moisture stress on the metabolism and growth of plants.

The effects of moisture stress on the rubber-bearing tissues of guayule are the aspects of this work which are perhaps of greatest interest. These tissues are the cortex, rays, pith, and certain regions of the secondary phloem; each is larger in treatment I (low moisture stress) than in treatment V (high moisture stress). It is interesting that in rubber-bearing tissues that are semimeristematic (as the cortex), and those derived from the cambium (the rays), cell size is not increased by low moisture stress but the number of cells is. In the pith, which is not meristematic, the effect of low moisture stress is to enlarge the tissue by enlarging the individual cells. The considerably larger size of plants grown under low moisture stress (5) indicates a much greater volume of each of these tissues than a comparison of cross-sectional areas would suggest. These observations on nursery seedlings corroborate and extend the findings of LLOYD (6) and ARTSCHWAGER (2) on field plants.

Summary

1. Sections of root-crown, young stem, and leaf of nursery plants of guayule

that had been subjected to three levels of moisture stress were examined anatomically.

2. Under the conditions of the experiment, moisture stress had no effect on the thickness of the cork, the width of the vascular rays, the length of cells in the cortex or epidermis, the width of cells in the cork or rays, or on the number of cells along a diameter of the pith.

3. Low moisture stress leads to increased cambial activity, resulting in greater xylem and phloem areas with correspondingly longer vascular rays. The diameter of the pith and the size of its cells are also greater under low moisture stress.

4. At the intermediate moisture stress, the development of xylem appears to have priority over the development of phloem.

5. High moisture stress leads to increased deposition of rubber and lignin and to considerable enlargement of the resin canals.

6. In general, low moisture stress leads to enlargement of the tissues of the plant with corresponding increases in rubber-bearing capacity. High moisture stress leads to the accumulation of the products of photosynthesis.

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EFFECT OF CHEMICAL TREATMENTS IN PROLONGING DORMANCY OF TUNG BUDS. II

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Introduction

Injury to the flowers by late spring frosts is one of the most serious hazards in commercial tung-oil production in the United States. Losses in small localized areas occur almost every season, and a partial to an almost total loss of crop occurred throughout the tung-growing belt in 2 of the 6 years 1938-1943, inclusive. Although it is known that the trees tend to compensate for such losses through the production of an extra heavy crop after a nonbearing year, average production over a period of time is probably reduced at least 25% by frost damage.

Numerous investigators (6-14) have reported that certain synthetic growth-regulating chemicals affect the rate of growth of the terminal and lateral buds of plants. WINKLEPLECK (12, 14) found that α -naphthaleneacetic acid in a water solution, when sprayed on floral buds of peach trees prior to blossoming, retarded full bloom for 11 days. Later, MITCHELL and CULLINAN (8) extended this study to include the effect of indole-3-acetic acid, indole-3-butyric acid, and α -naphthalene acetamide—in lanolin or oil emulsions—on the opening of vegetative and floral buds of the peach and pear. In their work the expansion of the floral buds was not retarded except as a result of injury, although the compounds were applied to attached and to excised

branches under different environmental conditions and to plant material which varied with respect to stage of development and physiological condition. In fact, under certain environmental conditions in the field they found that application of these substances may hasten the opening of floral buds. On the other hand, the growth of vegetative buds was consistently retarded as a result of the application of α -naphthaleneacetic acid.

In the tung tree, SELL *et al.* (10) observed in 1941 that dormancy was prolonged with α -naphthalene acetamide or indole-3-acetic acid and that there was a period of about 1 week during which injury to the buds on branches treated with the former by a frost of 28° F. would, according to FERNHOLZ (3), have been 5-15%, whereas on untreated branches it would have been 70-80%. In 1942 the study was extended to determine the effect of other organic compounds and also to determine whether ether and alcoholic extracts of tung buds contained any substance which would prolong dormancy. In many instances responses to the growth-regulating substances have been obtained through the use of a lanolin carrier (7-10). Since lanolin contains compounds of the steroid group and other organic substances, an effort was made to determine whether lanolin emulsion or some of the sterols and their palmityl esters in a partially hydrogenated vegetable-fat carrier might contain any biologically active substances that would prolong dormancy.

Recently, CLARK and KERNS (2) have established that flowering in pineapple

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could be induced in advance of the normal period—or considerably delayed—by the use of appropriate concentrations of such synthetic substances. Since the effect of concentration is such an important factor in controlling bud growth, the project of prolonging dormancy in tung was also extended to determine the effect of concentration of α -naphthalene acetamide and the effect of the date of its application.

In view of the injury resulting from application of growth-regulating substances reported by MITCHELL and CULLINAN (8) to buds of peaches and pears, a careful investigation was made to determine the extent of injury to the terminal tung buds.

Experimentation

EFFECT OF ORGANIC COMPOUNDS WITH VARIOUS FUNCTIONAL GROUPS

The term "tung buds" as used in this paper pertains only to flower buds, although leaf buds are also present in the flower cluster.

Thirty-one treatments were used, including the following as controls: untreated buds, Crisco (trade name for a partially hydrogenated vegetable fat) emulsion, and lanolin emulsion. In the twenty-eight other treatments, an emulsion—either of Crisco or of lanolin—was used as a carrier for the various substances to be tested. To compensate for the error due to the variation in data of bloom on individual seedling trees, the symmetrical incomplete block design as described by GOULDEN (5) was used. Each plot consisted of the buds (20–133) on a single branch. Six such branches were utilized on each of thirty-one trees 16 years old, making in all about 186 plots and providing six replications of each of the thirty-one treatments.

The six branches on a single tree constitute a "block" (235–387 buds); and in the symmetrical incomplete block design, adjustment for variations between blocks is accomplished by an arrangement of the treatments assigned to each block (in this case to each tree). For example, the six replications of treatment A were located on one branch on each of six trees, and since there were six experimental branches on every tree, the six trees on which treatment A was located had in the aggregate thirty-six experimental branches: six had been used for treatment A and the remaining thirty were available for other treatments; hence it was possible to apply the remaining thirty treatments (B, C, D, etc.), one on each of the remaining thirty branches. Thus it was possible to calculate from the data for these six trees (a) the average response to treatment A, and (b) the average response to all treatments. The blocks—that is, the groups of treatments assigned to each tree—are so arranged that what has just been described for treatment A holds true for every other treatment, and it is possible to compare the response to each of the thirty-one treatments in turn with the average response to all treatments on the same trees. Thus a common denominator that is independent of individual tree variations is provided for adjustment of the actual responses.

The various substances were mixed in a lanolin emulsion according to the method of WINKLEPLECK (13). The basic formula is lanolin 38.0 gm., stearic acid 7.5 gm., triethanolamine 2.7 gm., and water 100 gm. The substances to be tested were dissolved in the minimum quantity of dioxane and then stirred into the lanolin emulsion by means of a Hamilton-Beach dispersion apparatus. Concentrations of 0.50% in the emulsion

were prepared with the organic compounds and of 16% with the extracts of tung buds. For the preparation of a Crisco emulsion, Crisco was substituted for lanolin in the WINKLEPLECK formula.

Four applications of these various materials were made on February 12, 26, March 11, and 20, 1942, to the buds of individual branches on the various trees by means of a small varnish brush.

this by the total number of buds. For example, on the branch of tree 5 that had been treated with lanolin emulsion alone, there were on April 1 seven buds in stage 1, thirteen in stage 2, one in stage 3, and four in stage 4. Thus there were twenty-five buds, the sum of the products was fifty-two, and the average rating was 2.08. This figure (2.08) indicates that the average bud on the branch used as an

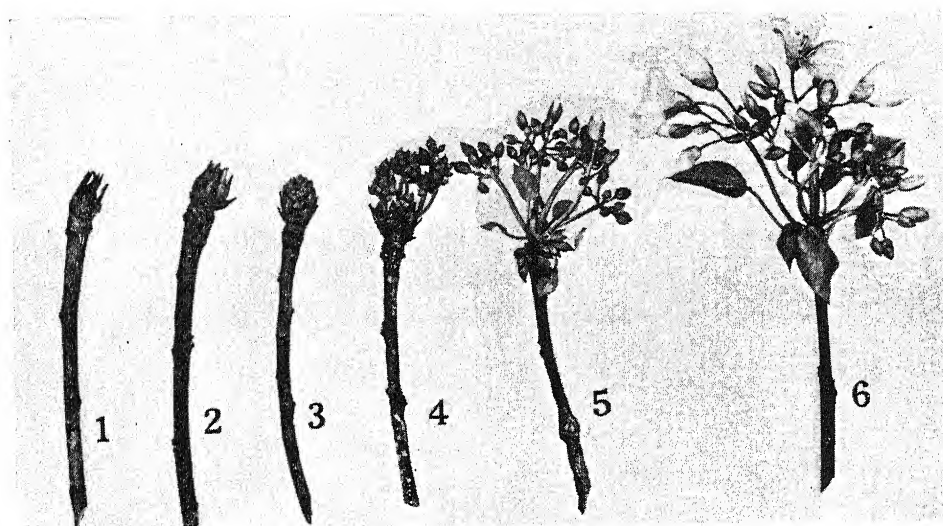


FIG. 1.—Tung buds at six stages of development

As in a previous study by SELL *et al.* (10), development of the buds from dormancy to post-bloom was divided into seven stages. The buds shown in figure 1 illustrate the midpoints of the first six stages. Stage 7 (not illustrated) comprises the terminal on which petals had begun to fall. When growth began, the stage of development of the living buds was assigned a numeral, rating from 1 (dormant) to 7 (post-bloom). The average rating of the stage of development of the buds on each branch was found by multiplying each numerical rating by the number of buds at that stage, adding the products, and dividing

example had developed slightly beyond stage 2 (fig. 1). Similar ratings were calculated for the five other control branches treated with lanolin emulsion, the average for all six branches was statistically adjusted as previously explained to compensate for the individual tree variation, and the adjustment rating 1.65 was entered in table 1 as the effect of lanolin-emulsion control on April 1.

As the season advanced, considerable injury to the buds was observed, and on May 30 the dead buds on each branch were counted. These data were expressed as percentages and have been analyzed

statistically after transformation to angles—as recommended by BLISS (1)—to obtain the F value (table 1). Since transformed data are of little interest, actual percentages of dead buds are shown in table 1. No standard error appropriate

anhydride, 2, 4-dinitro-1-naphthol-7-sulfonic acid, para-aminobenzoic acid, 8-hydroxyquinoline, and ether and alcoholic extracts of tung buds. These substances neither caused greater injury to the buds nor retarded development more

TABLE 1

ADJUSTED RATINGS OF STAGE OF DEVELOPMENT (FIG. 1) AND PERCENTAGE INJURY FOR TUNG BUDS FOLLOWING FOUR APPLICATIONS OF 0.50% CONCENTRATIONS OF ORGANIC COMPOUNDS IN LANOLIN EMULSION (EXCEPT WHERE CRISCO IS INDICATED) MADE FEBRUARY 12, 26, MARCH 11, AND 20, 1942

TREATMENT	MARCH 19	MARCH 22	APRIL 1	APRIL 7	APRIL 13	APRIL 20	APRIL 27	DEAD BUDS AS OF MAY 30 (%)
	Adjusted ratings of stage of development							
Untreated control.....	1.42	3.17	3.98	5.66	6.77	7.00	7.00	0.00
Lanolin-emulsion control.....	1.02	1.16	1.65	2.99	4.21	5.75	6.82	7.49
Naphthalene.....	1.00	1.35	1.76	3.53	4.59	6.43	6.98	3.16
α -Naphthoic acid.....	1.00	1.00	1.06	2.71	4.02	5.84	7.00	12.52
α -Naphthaleneacetic acid.....	1.00	1.14	1.29	1.92	2.78	3.74	4.97	52.49
α -Naphthalene acetamide.....	1.00	1.00	1.00	1.61	2.90	3.74	5.22	38.52
α -Naphthalene thioacetamide.....	1.00	1.01	1.23	1.85	2.88	3.74	4.52	41.65
Indole-3-acetic acid.....	1.00	1.03	1.20	2.47	3.39	4.54	5.98	16.94
Phytosterol palmitate.....	1.03	1.09	1.29	2.85	4.37	5.74	6.91	12.91
Phytosterol.....	1.02	1.52	2.22	3.95	5.31	6.55	6.93	7.45
Cholesterol.....	1.08	1.32	1.60	3.29	4.36	6.30	7.00	7.33
Cholesterol palmitate.....	1.00	1.10	1.38	2.76	3.84	5.24	6.88	18.00
Crisco and phytosterol palmitate....	1.03	1.50	1.99	3.70	5.12	6.83	7.00	1.33
Crisco-emulsion control.....	1.04	1.64	2.37	4.02	5.53	6.61	7.00	2.65
Crisco, cholesterol palmitate, and α -naphthalene acetamide.....	1.01	1.00	1.06	1.50	3.30	4.36	6.03	20.52
F for treatments*.....	4.15	8.72	9.90	9.36	9.78	7.64	3.94	5.70†
Standard error mean difference....	0.054	0.20	0.26	0.37	0.38	0.48	0.49	†
Least difference significant at 0.05...	0.11	0.40	0.51	0.73	0.75	0.95	0.97
Least difference significant at 0.01...	0.14	0.52	0.68	0.97	1.00	1.26	1.28

F required at 0.001 = 2.40

* Value of F determined by analysis of data for thirty-one treatments, including seventeen not shown in table.

† Value of F obtained by analysis of transformed values.

‡ No standard error appropriate to whole range of percentages of dead buds can be calculated; for values approaching zero the error is smaller than for percentages approaching 50.

to the whole range of percentages shown can be calculated.

Data will not be presented for lanolin emulsions of the following compounds: 1,2-azonaphthalene, 2,4-dinitro-6-cyclohexylphenol, phenylthiohydantoic acid, sulfanilamide, ethyl carbamate, 3,5-dinitrosalicylic acid, sulfanilic acid, phenylisothiocyanate, pyridine, maleic

than did the lanolin-emulsion control. Data for the average stage of development at weekly intervals and the percentage injury to the buds subjected to the remaining fifteen treatments are shown in table 1. Certain of these treatments were also without significant effect; but, owing to the chemical relationships of the compounds to either

lanolin or naphthalene and its related compounds, the data are included in the table.

The lanolin-emulsion control retarded blossom development. By April 1, terminal buds on the untreated control branches had attained stage 4, in which they are rather susceptible to freezing injury, while those treated with the lanolin-emulsion control did not reach this stage until April 13—12 days later. According to FERNHOLZ (3), 70–80% of the pistillate flowers on terminals in stage 4 would be either injured or killed by a temperature of 28° F. However, the retardation appears to be the result of injury to the buds, of which 7.49% were killed.

In the series of naphthalene compounds (table 1) it was noticed that naphthalene produced no greater response than did the lanolin-emulsion control. A slight retarding effect was noticed with α -naphthoic acid on March 26, April 1 and 7, but it was only on April 1 that the response differed significantly from the lanolin-emulsion control. The α -naphthaleneacetic acid and its two derivatives gave the greatest response of any of the substances tested. On April 7, the control buds (untreated) and those treated with lanolin-emulsion control were nearly in stages 6 and 3, respectively, while those emulsions fortified with the α -naphthaleneacetic acid and its derivatives reached stage 3 on April 13, 6 days later. It was evident, however, that the increased retardation of development by α -naphthaleneacetic acid and its derivatives was associated with increased injury to the buds, since 38–52% of these were killed.

The indole-3-acetic acid was slightly less effective than the α -naphthaleneacetic acid derivatives. This may be due to the fact that the indole-3-acetic acid

is a more unstable compound than the latter. Preparations of indole-3-acetic acid stored in amber bottles have been found to deteriorate on standing at room temperature in the laboratory for about 12 months. Purification of 3 gm. of this stored material yielded only 1 gm. having the correct melting point upon recrystallization from chloroform. Examination of the mother liquor showed that the remainder of the material had decomposed beyond recovery.

The lanolin emulsion—fortified, respectively, with cholesterol palmitate found in lanolin and phytosterol palmitate found in the wax of tung buds (table 1)—produced no response as compared with the lanolin emulsion without the growth-regulating substances. There is a slight indication that phytosterol palmitate in a Crisco emulsion retarded development early in the season more than the Crisco-emulsion control, but at no date does the difference attain statistical significance. However, phytosterol (Eastman grade) in lanolin emulsion produced significantly less effect in retarding bud expansion on April 1, 7, and 13 than did lanolin-emulsion control, but such differences were not significant on the other four dates. The Crisco-emulsion control prolonged dormancy about 1 week. Crisco emulsion fortified with cholesterol palmitate plus α -naphthalene acetamide yielded a significant response in prolonging dormancy but killed 20.52% of the buds. On March 26, the buds to which the Crisco-emulsion control had been applied were almost midway between stages 1 and 2, and this same stage was reached on April 7 by the buds treated with the Crisco emulsion fortified with cholesterol palmitate plus α -naphthalene acetamide. Since it has been shown previously that fortifying Crisco emulsion with chole-

terol palmitate did not increase its effect on dormancy, the response just noted can be attributed to the α -naphthalene acetamide.

The data in table 1 show that in all instances retardation of development of buds was associated with injury. The substances that prolonged dormancy for the longest time also killed the most buds. The palmityl esters of cholesterol and phytosterol produced a more moderate effect on growth and injured fewer buds. It is concluded that a 0.50% emulsion of the growth-regulating substance used in a lanolin emulsion is not a practical means of prolonging dormancy in terminal buds of the tung tree.

EFFECT OF CONCENTRATION AND DATE
OF APPLICATION OF α -NAPHTHA-
LENE ACETAMIDE

Two experiments were set up, one to determine the effect of different concentrations of α -naphthalene acetamide in single applications and the other to determine the effect of the same concentration of this substance used in schedules of repeated applications.

In the first experiment, lanolin emulsion made according to the formula of WINKLEPLECK (13) was used in preparing three concentrations of α -naphthalene acetamide—0.50, 0.25, and 0.01%. On each of four dates (February 12, 26, March 11, and 20) these dilutions were applied by means of a small varnish brush to the buds of previously untreated branches of twenty-four seedling trees. Each treatment was replicated on six branches. Other branches on the same tree were left untreated as controls.

In the second experiment, the same concentrations of α -naphthalene acetamide were used in four schedules as follows: (a) four applications each on February 12, 26, March 11, and 20; (b)

three applications on February 26, March 11, and 20; (c) two applications on March 11 and 20; (d) one application on March 20.

Under the same environmental conditions, different seedling trees vary widely in date of bloom. In order to correct for this source of error, the two experiments were arranged on a factorial basis, as described by FISHER (4). Each concentration was used on every date or schedule, with a control. These separate controls (untreated buds), although identical, are listed as separate treatments, thus giving sixteen treatments in each experiment. This permits the use of a simple yet adequate experimental design.

There were six replications of the sixteen treatments in each experiment. The degrees of freedom representing the interaction between concentration and date or schedule of application were partially confounded, thus permitting the sixteen treatments of each replication to be broken down into four blocks of four treatments each. The four branches of each of the twenty-four trees constituted a block. Thus, the variation in date of bloom of the individual tree had no effect upon the over-all responses (a) to date or schedule of application or (b) to concentration. This design also reduced to a minimum the effect of tree variation on the data for each specific combination of date with concentration.

At weekly intervals, beginning March 19, the stage of development of each bud on every branch was recorded as previously described. The average rating of the stage of development for each treatment on each date was calculated. The data show that the buds on control branches were about to blossom on April 8, therefore a statistical analysis has been made of the data for all treatments as of this date. The data on stage

of development and percentage of dead buds as affected by concentration at a certain date, and those for schedules of concentrations were applied on February 12 (table 2) had developed into stage 3 (fig. 1) by April 8, whereas—by repeated

TABLE 2

AVERAGE RATINGS OF STAGE OF DEVELOPMENT (FIG. 1) OF LIVING TUNG BUDS ON APRIL 8 AND PERCENTAGE OF DEAD BUDS AS OF MAY 30 IN RELATION TO CONCENTRATION, TIME, AND FREQUENCY OF APPLICATION OF α -NAPHTHALENE ACETAMIDE IN LANOLIN EMULSION

DATES OF APPLICATION	PERCENTAGE CONCENTRATION OF α -NAPHTHALENE ACETAMIDE								
	0.50		0.25		0.01		Control		Average (r)
	Dead buds (%)	Stage of development (r)*	Dead buds (%)	Stage of development (r)	Dead buds (%)	Stage of development (r)	Dead buds (%)	Stage of development (r)	
Experiment 1, single applications									
2/12.....	3.28	19.6	3.26	4.0	4.24	0.0	5.48	4.07
2/26.....	35.7	3.38	16.2	3.60	0.3	5.07	0.0	5.41	4.37
3/11.....	19.7	3.96	17.4	4.53	0.8	5.67	0.4	5.44	4.90
3/20.....	7.8	5.50	0.0	5.74	1.3	5.23	5.52	5.50
Average.....	4.03	4.28	5.05	5.46	4.71
Experiment 2, repeated applications									
2/12, 2/26, 3/11, 3/20 (schedule 1).....	67.5	1.52	59.4	1.60	19.0	3.10	5.42	2.91
2/26, 3/11, 3/20 (schedule 2).....	63.4	2.07	39.4	2.05	11.9	4.46	0.0	5.17	3.44
3/11, 3/20 (schedule 3).....	17.6	5.14	29.7	4.87	6.7	4.40	0.8	5.45	4.96
3/20 (schedule 4).....	10.9	5.34	1.6	5.48	1.6	5.33	0.3	5.60	5.44
Average.....	3.52	3.50	4.32	5.41	4.19

	TREATMENTS		MEANS	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Standard error of difference between ratings.....	0.34	0.32	0.17	0.16
Least difference significant at 0.05.....	0.68	0.64	0.34	0.32
Least difference significant at 0.01.....	0.90	0.85	0.45	0.43
Least difference significant at 0.001.....	1.18	1.11	0.59	0.55

No error applicable to whole range of percentage dead buds can be given.

* r=rating.

repeated applications, are summarized in table 2.

It is evident that the effects of repeated applications are cumulative. Buds to which the 0.50 or the 0.25%

applications of the same concentrations (schedule 1, table 2)—development had been more effectively retarded and the buds were midway between stages 1 and 2. In general, however, repeated appli-

cations on all dates killed a much higher percentage of the buds than did single applications. These data add to the previous evidence that the prolonging of dormancy obtained in this way is associated with injury to the buds.

It is also evident that, to be effective, applications must be made early. Schedule 1, consisting of four applications (table 2), was the most effective in prolonging dormancy. No outstanding effect on date of bloom was produced by the two single-application tests made on March 20. The injury to the buds from applications made on this date was less than that produced by earlier applications. Although the two highest concentrations of the single applications of α -naphthalene acetamide applied on March 11 produced a statistically significant delay in date of bloom, this difference is not of practical importance since the buds required only about 1 day of growth to pass from stage 4 to stage 5.

For all dates and schedules of applications, without exception, there were no statistically significant differences in effectiveness in prolonging dormancy between 0.50 and 0.25% concentration of the α -naphthalene acetamide in lanolin emulsion. In the case of the single applications of February 26 and of March 11, the 0.50% concentration appears to have been slightly more effective than the 0.25%, but the difference is within the range of experimental error. The number of dead buds is somewhat greater with 0.50 than with 0.25%.

The 0.01% α -naphthalene acetamide when applied on February 12 was approximately one-half as effective as either the 0.50 or the 0.25% but produced no effect when applied on February 26. In schedule 1, consisting of four applications beginning February 12, the 0.01% concentration again proved

to be approximately one-half as effective as either the 0.50 or 0.25% concentration. In schedules 2 and 3 of the repeated applications, this 0.01% concentration was less effective than in schedule 1. It produced very little injury to the buds, excepting in schedules 1 and 2.

In 1942, the period of dormancy² in tung buds terminated about mid-March, and the optimum time of application was about 1 month earlier. Applications had to be repeated to attain maximum effectiveness. Of the concentrations used in this experiment, 0.25% of α -naphthalene acetamide in lanolin emulsion proved the most effective. Injury to the buds occurred wherever blossoming was delayed, however, and no combination of concentration with date of application was found satisfactory or practicable for delaying blossoming.

Summary

1. Dormancy in tung buds was prolonged by treatment with lanolin emulsion alone, the buds remaining about 12 days longer in a stage not injured by a temperature of 28° F. This treatment killed 7.5% of the buds. Crisco emulsion was approximately one-half as effective as the lanolin emulsion and produced less injury.

2. Alpha-naphthaleneacetic acid, two of its derivatives, and indole-3-acetic acid were the only compounds used in lanolin emulsion that prolonged dormancy more than did the lanolin emulsion alone. The percentages of dead buds resulting from these more effective treatments ranged from 12.5 to 52.5, whereas the percentage for the lanolin emulsion was 7.5.

² The term "dormancy" as used in this paper does not refer to the rest period but rather to that period during which no development of the buds was observed in the field.

3. Repeated applications of 0.50 and 0.25% of α -naphthalene acetamide in lanolin emulsion were more effective than single applications in prolonging dormancy and also killed more buds. The injury was slightly greater with the 0.50% concentration. No significant differences in response were noticed between the 0.50 and 0.25% concentrations. The 0.01% concentration was approximately one-half as effective as the 0.50 or the 0.25% and produced considerably less injury. The indications are

that two applications of 0.25% α -naphthalene acetamide in a lanolin emulsion made about 30 and 15 days prior to bud expansion would give best results in prolonging dormancy. Since considerable injury occurred whenever blossoming was materially delayed, no combination of concentration with date of application was found practical for orchard use.

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2,4-DICHLOROPHENOXYACETIC ACID AS A DIFFERENTIAL HERBICIDE

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Introduction

In studying plant responses to growth-regulating substances, it has since 1936 been observed repeatedly that some of these substances are toxic to plant tissues when applied in relatively large amounts. Thus, localized application of lanolin paste containing 2% or more of indoleacetic acid or of naphthoxyacetic acid often results in the death of tissues near the treated regions; and in connection with vegetative propagation of plants, overtreatment with these compounds has often resulted in greatly reduced growth or in death of the cuttings. For some years, responses to growth regulators, which were associated with decrease in plant growth, were considered disadvantageous in crop improvement, and they received relatively little attention. However, KRAUS (3) in 1941 proposed that the growth-inhibiting properties of these compounds be utilized in connection with weed control.

Recently, experimental evidence was obtained indicating that 2,4-dichlorophenoxyacetic acid, a potent substance whose activity was discovered by ZIMMERMAN (6), possesses herbicidal properties when applied in a solution of Carbowax (5) or when dispersed as a liquefied-gas aerosol (1, 2).

In April, 1944, experiments were undertaken at Beltsville, Maryland, to determine the sensitivity of various common weeds to treatment with 2,4-di-

chlorophenoxyacetic acid. These experiments have since been extended in order to determine the possibility of using the acid as a differential herbicide, and the results are reported here.

Methods

Two methods of application were used, aqueous sprays and the liquefied-gas aerosol. The aqueous spray solutions were prepared by dissolving the required amount of the acid in Carbowax 1500 (polyethylene glycol), then adding this solution, with stirring, to the required amount of tap water. The Carbowax served as a carrier and spreading agent (5) and was added in amounts sufficient to give a 0.5% solution. Sprays were applied to plants or small plots with a precision sprayer of 1-quart capacity and carrying an air pressure of 60-100 pounds, or by means of a knapsack 3-gallon sprayer when somewhat larger plots were treated.

In dispersing the acid as an aerosol, a metal cylinder equipped with outlet and valve was evacuated. One hundred fifty grams of dimethyl ether and 25 gm. of engine oil (SAE 40) containing 3.15 gm. of 2,4-dichlorophenoxyacetic acid was drawn into the vessel. The mixture was then dispersed as an aerosol by releasing the valve, and treatments were applied by directing the mist toward the plants for several seconds. In some instances the plants were first covered with a cloth tent and the aerosol released within the enclosure, where it was allowed to remain for a period of several seconds.

PRELIMINARY EXPERIMENTS.—In preliminary experiments under field condi-

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tions, weeds on plots of ground 2×2 feet were treated with the aerosol and the behavior of the plants later compared with that of others of like species on an adjacent untreated plot. In other exploratory experiments, weeds of uniform size and in the seedling stage were transplanted from the field to 4-inch pots and grown under greenhouse conditions until well established. The species used included *Plantago lanceolata*, *P. major*, *Datura stramonium*, and *Capsella bursa-pastoris*. Aerosol treatments were applied to these plants in varying amounts and their subsequent behavior noted.

EXPERIMENTS WITH DANDELION.—On September 2, an area of Kentucky bluegrass lawn 32.5 feet long and 13 feet wide was selected in which an even, dense stand of dandelion (*Taraxacum officinale*) was growing. Six adjacent plots 6.5 feet square were laid out, two replications for each of three treatments. The following treatments were applied: (a) untreated control, (b) 500 p.p.m. of 2,4-dichlorophenoxyacetic acid applied as a spray at the rate of 4 gallons per 1000 square feet, and (c) 1000 p.p.m. of the acid applied in the same manner and rate. The spray treatments were repeated 26 days after the initial treatment. On September 21, Milorganite fertilizer was applied at the rate of 20 pounds per 1000 square feet of lawn surface. During the experiment the lawn was cut at regular intervals and given usual lawn care.

EXPERIMENTS WITH PLANTAIN.—On August 28, an area of Kentucky bluegrass lawn heavily infested with narrow-leaf plantain (*Plantago lanceolata*) was selected for uniformity of both grass and plantain. Three parallel tiers of plots 6 feet square were laid out in this area, with twenty plots in each tier—a total of sixty plots. A 1-foot border was left around each plot. The three tiers of plots

were separated into four equal blocks or replicates, each being five plots long and three plots wide. Aqueous sprays containing 2,4-dichlorophenoxyacetic acid in concentrations of 0, 125, 250, 500, and 1000 p.p.m. were applied. Five plots in each block were selected at random, and on August 28 four of these received a single spray treatment at each respective concentration level. Five other plots in each block received two applications of the respective solutions, the first on August 28 and the second on September 7. The five remaining plots in each block were reserved for treatment during the spring season.

All spray applications were made by means of a 3-gallon knapsack sprayer at the rate of approximately 4 gallons per 1000 square feet. The grass was cut when needed during the experiments and the lawn given usual care.

Results

PRELIMINARY EXPERIMENTS.—Plants of *Datura*, *Plantago*, and *Capsella* transplanted from outdoors and grown under greenhouse conditions during April were found to be very sensitive to application of 2,4-dichlorophenoxyacetic acid. Seedlings of *Datura* were killed by aerosol treatments of 5 seconds' duration. Exposure for 1 second to the aerosol resulted in the death of plants of *Plantago* (fig. 1) and *Capsella*. These treatments were applied after the plants had developed visible flower buds but before any flowers had opened. Within 48 hours following treatment the plants showed severe leaf-curl, and—in the case of *Datura*—stem bending. There was no evidence of burning of the leaves, but growth of the plants was inhibited, the leaves became light green in color, and within 3 weeks the sprayed plants were dead. European bindweed (*Convolvulus*

arvensis) grown from seed during early spring under greenhouse conditions was also killed by a 5 seconds' exposure to the aerosol.

In preliminary field experiments, treatment applied on August 11 to ma-



FIG. 1.—Eradication of narrow-leaf plantain (*Plantago lanceolata*) with 2,4-dichlorophenoxyacetic acid applied by aerosol method: 1, untreated; 2, exposed to the aerosol for 1 second; 3, for 5 seconds; and 4, for 20 seconds. Photographed approximately 1 month after treatment.

ture dandelion plants resulted in complete killing by August 29. Likewise, applications of aqueous sprays containing 1000 p.p.m. of the acid resulted in almost complete eradication of several hundred dandelions from a lawn area 35 feet long and 10 feet wide. Two plots containing forty-five to fifty plants each of ragweed were treated on August 12 by means of the aerosol method, while two similar adjacent plots received a thorough coverage with an aqueous spray containing 800 p.p.m. concentration of the acid. Periodic examination of these plots indicated that application of 2,4-dichlorophenoxyacetic acid, both as an aerosol and in water, had resulted in complete inhibition of vegetative growth. Maturation of flowers was inhibited as the result of both methods of treatment, and the shedding of pollen was prevented. On August 29, all the plants in the aerosol plots were dead.

Ninety per cent of the plants that received the aqueous-spray treatment were dead at this time, and the remainder were severely distorted. After death of the ragweed, volunteer crabgrass present at the time of treatment rapidly covered bare areas in all plots.

EXPERIMENT WITH DANDELION.—A more detailed experiment was undertaken, as previously described, to determine the effectiveness of 2,4-dichlorophenoxyacetic acid when used to eradicate dandelion plants in a Kentucky bluegrass lawn. The spray treatments listed in table 1 were applied to the treated plots on September 2 and the original number of dandelions per plot determined 3 days later. On the third day following treatment, the plants sprayed with the acid were severely twisted and had turned from dark green to a pale yellowish green color.

TABLE 1

ERADICATION OF DANDELION IN KENTUCKY BLUEGRASS SOD BY AQUEOUS SOLUTIONS OF 2,4-DICHLOROPHENOXYACETIC ACID AT 500 AND 1000 P.P.M. CONCENTRATIONS. FIRST SPRAY APPLICATION ON SEPTEMBER 2; SECOND ON SEPTEMBER 28

SPRAY CONCENTRA- TION (P.P.M.)	NO. OF DANDELION PLANTS PER PLOT (AV. OF 3 PLOTS)			PER CENTAGE ORIGINAL PLANTS KILLED	
	Sept. 7	Sept. 26	Oct. 19	Sept. 26	Oct. 19
Unsprayed control.	118.0	213.5	215.0	0	0
500.....	94.6	22.0	3.0	75.6	95.8
1000.....	117.0	4.6	1.5	96.0	98.3

During an interval of 24 days following treatment, the 500 p.p.m. spray reduced the original number of dandelion plants by 75.6%, while 1000 p.p.m. reduced the dandelion population by 96.0% (table 1; fig. 2). The number of

dandelion plants in control plots more than doubled during the same interval, owing to unrestricted germination of the seeds. Later examination showed that many of the dandelions killed by the spray treatments had completely disintegrated as the result of attacks by soil organisms. Apparently, application of

killing as obtained previously by a single application of a spray containing 1000 p.p.m. of the acid (table 1). On October 19, the newly planted grass had germinated and was growing vigorously. The bare areas had been almost completely covered with grass in the reseeded portions of the treated plots.

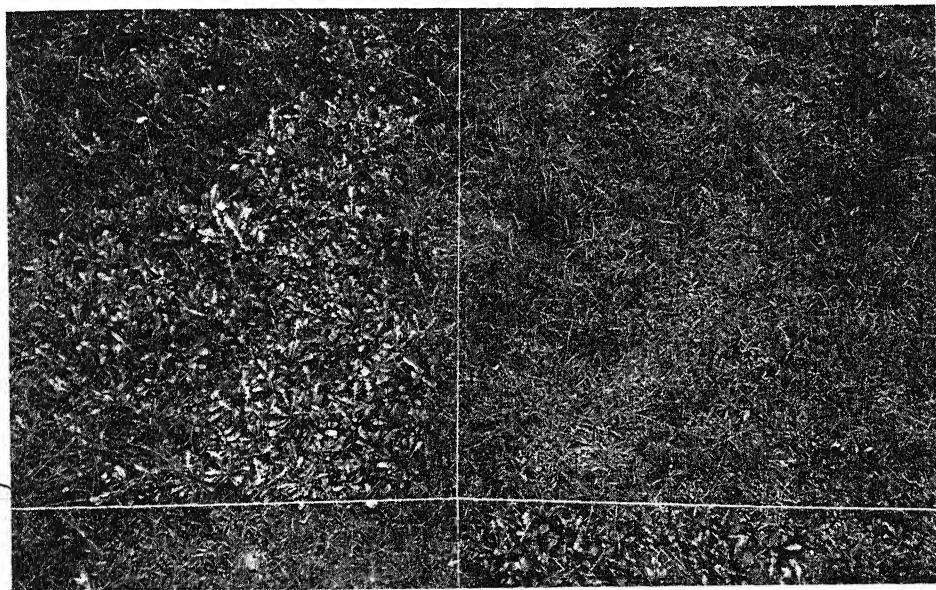


FIG. 2.—Eradication of dandelion from Kentucky bluegrass sod by spraying at rate of 4 gallons per 1000 square feet of sod with aqueous solutions of 1000 p.p.m. concentration of 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax. Sprayed plot at right; untreated control plot at left. Spray applied September 2 and plot photographed 24 days later.

the acid did not greatly inhibit the subsequent development of microorganisms in the tissues after the plants died.

Eradication of the dandelions left numerous bare areas, but the grass continued to grow following treatment, and regular weekly mowings were necessary to maintain it at a height of $1\frac{1}{2}$ inches. The plots were resprayed and one-half of each plot was reseeded on September 28. Dandelion counts were again made on October 19. Retreatment with the 500 p.p.m. concentration resulted in approximately the same percentage of

EXPERIMENT WITH NARROW-LEAF PLANTAIN.—On August 28, experiments were undertaken, as previously described, to determine the effectiveness of 2,4-dichlorophenoxyacetic acid when used to eradicate narrow-leaf plantain growing in an area of well-established lawn. Heavily infested plots sprayed twice with a solution containing 500 p.p.m. of the acid, or that received either one or two applications of 1000 p.p.m., were practically free of plantain 3 weeks after treatment (94.0–99.0% eradicated). There was no appreciable change in the

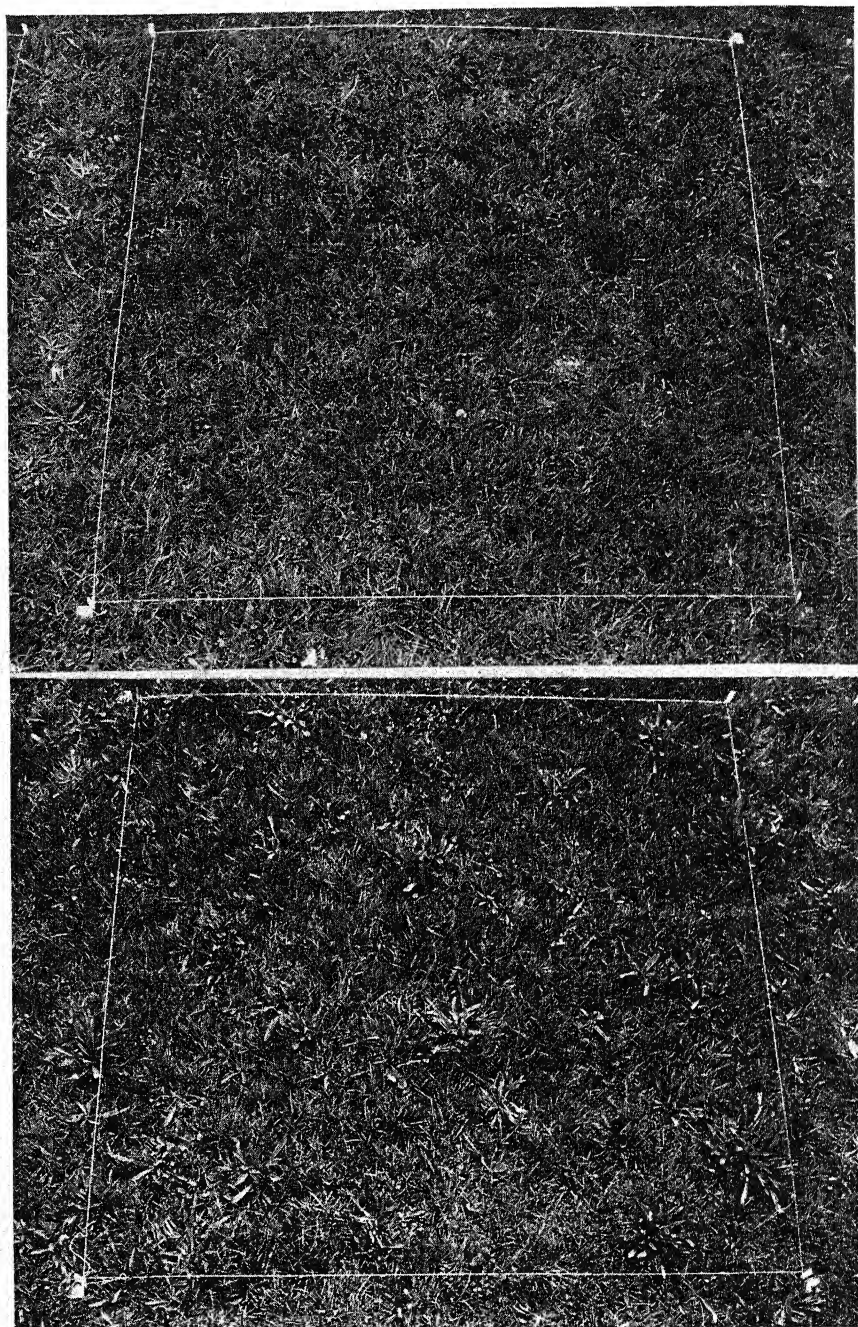


FIG. 3.—Eradication of narrow-leaf plantain from Kentucky bluegrass sod. Above: aqueous solution of 1000 p.p.m. concentration of 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax. Below: untreated control plot. Spray applied August 28 and plots photographed 23 days later.

population of plantain in untreated plots during the 52 days immediately following the first treatment, at which time final readings were made (table 2; figs. 3, 4). Lower concentrations (125 and 250 p.p.m.) were less effective, and some of the injured plants appeared to recover in these treatments. It is noteworthy that a single treatment at the 500 p.p.m.

TABLE 2

EFFECT OF ONE AND OF TWO AQUEOUS SPRAY APPLICATIONS OF VARIOUS CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID IN ERADICATING NARROW-LEAF PLANTAIN IN PLOTS 6X6 FEET OF KENTUCKY BLUEGRASS SOD

SPRAY CONCENTRATION (P.P.M.)	NO. OF APPLICATIONS*	NO. OF PLANTAIN PLANTS PER PLOT (AV. OF 4 PLOTS)			PERCENTAGE ORIGINAL PLANTS KILLED	
		Original count on Aug. 28	Count on Sept. 29	Count on Oct. 19	Sept. 29	Oct. 19
Unsprayed control..	0	62.4	66.1	69.7	0	0
125.....	1	68.2	69.0	64.0	0	0.6
	2	58.7	42.7	41.0	22.3	20.9
250.....	1	52.5	47.2	39.5	10.0	24.7
	2	54.2	18.5	26.5	65.9	51.1
500.....	1	53.7	14.5	17.2	73.0	67.6
	2	63.0	3.5	0.7	94.0	98.8
1000.....	1	45.5	2.0	3.0	95.6	93.4
	2	50.2	0.5	0.2	99.0	99.5

* Single spray treatment on August 28; duplicate sprayings on August 28 and September 7.

concentration killed only 67.6% of the plantain, while the double application at this concentration was as effective as the 1000 p.p.m. spray applied either once or twice. These data indicate that, although thorough coverage may be of considerable importance, it was also necessary to deposit the acid on the weed foliage in sufficient amounts per unit surface area to bring about adequate selective killing. Apparently, repeated sprayings with relatively low concentrations would give about the same effects as fewer applications of sprays containing relatively high

concentrations, provided approximately equivalent amounts of the acid are deposited in each instance. Further experiments are necessary to determine which type of spray treatment is more desirable. At present, grass in all the plots is growing vigorously. Grass that received a spray containing 1000 p.p.m. is a somewhat darker, richer green than that in other plots which received the more dilute sprays. Intensification of green col-

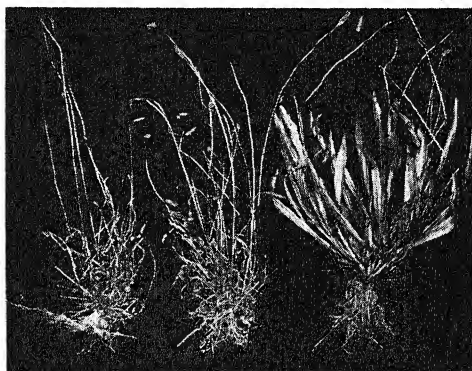


FIG. 4.—Effect of 2,4-dichlorophenoxyacetic acid (1000 p.p.m. spray) on mature field-grown plants of plantain. Left: two applications, August 29 and September 7. Center: single application, August 29. Right: untreated control.

oration has also been noted with other experimental plants, such as bean and tomato, when these were sprayed with solutions containing growth-regulating substances. Whether in this instance it may or may not be desirable has not yet been determined.

A number of common weeds other than those already listed occurred in the treated plots in many of the experiments. The following were very sensitive and readily killed by aqueous sprays at 500-1000 p.p.m. concentrations: woodsorrel (*Oxalis* sp.), chickweed (*Stellaria media*), pigweed (*Amarantus retroflexus*), knotweed (*Polygonum avicular*), and morning glory (*Convolvulus sepium*).

Other weeds—such as oxeye daisy (*Chrysanthemum leucanthemum* var. *pinnatifidum*), yarrow (*Achillea millefolium*), broad-leaf plantain (*Plantago major*), sheep sorrel (*Rumex acetosella*), dock (*Rumex obtusifolius*), as well as two

United States Golf Association. These concern the eradication of Dutch white clover from bluegrass and bentgrass sods. In these experiments clover was found to be very sensitive to aqueous sprays containing 2,4-dichlorophenoxy-

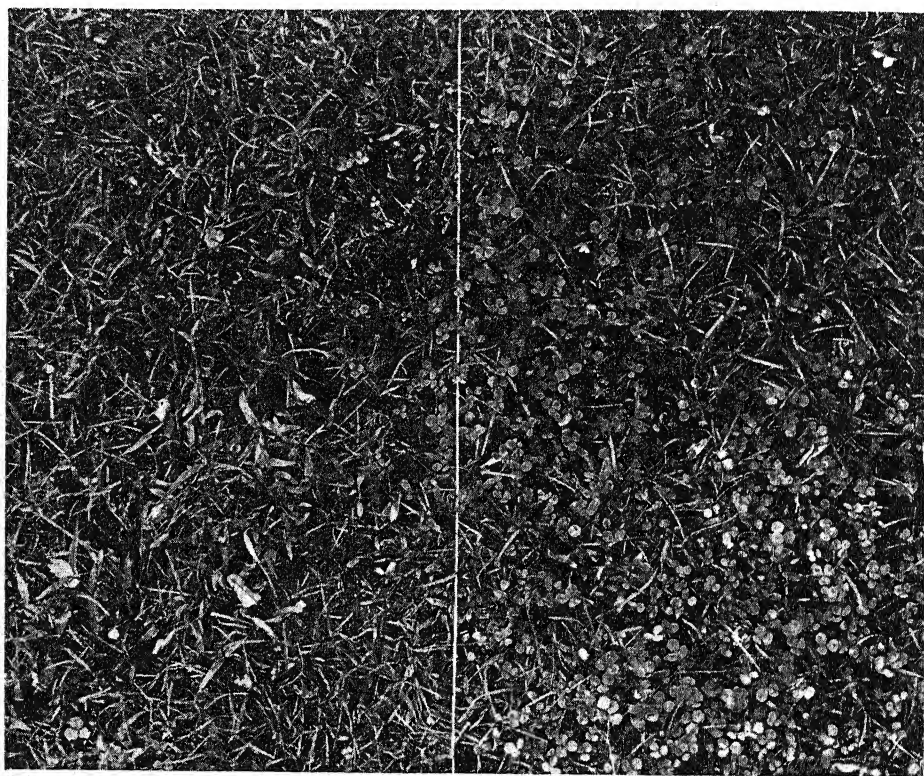


FIG. 5.—Eradication of Dutch white clover from Kentucky bluegrass sod. Left: 1000 p.p.m. concentration of 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax applied as spray in water. Right: untreated control. Bluegrass and crabgrass in treated plot apparently uninjured.

species of *Rubus*—a blackberry and a trailing dewberry—appeared to be more resistant to the sprays and tended to recover from single applications at 1000 p.p.m. concentration. Preliminary results, however, indicate that these weeds may be killed by repeated sprays at this concentration.

A series of similar experiments reported elsewhere (4) has been in progress in co-operation with the Greens Section,

acetic acid (fig. 5). In the same series of experiments, shiny pennywort (*Hydrocotyle rotundifolia*), which had been crowding out the bluegrass in a lawn for a period of several years, was found to be even more readily killed than clover. A single spray with the 500 p.p.m. concentration of the acid at 5 gallons per 1000 square feet resulted in its complete eradication from bluegrass sod, without apparent injury to the grass.

Discussion

Chemicals such as sodium chlorate, sodium thiocyanate, and various arsenical preparations have been used extensively for weed eradication during the last decade. These compounds are extremely toxic to plants when applied in relatively large amounts (1-10% solutions), and their killing action is generally characterized by a localized burning effect which may later extend through the plant for some distance from the point of contact. In contrast, 2,4-dichlorophenoxyacetic acid is effective in concentrations of approximately 0.025-0.1%. It does not cause rapid superficial burns, but it brings about morphological and physical responses at the point of application or in parts of the plant at some distance from this region. These growth responses usually occur soon after treatment (within 24 hours), and apparently before the concentration of the acid or of its derivatives reaches a lethal level in the plant, and they are characterized by distorted stem and leaf growth, by inhibition of bud growth (particularly the terminal ones), and sometimes by the formation of galls or roots in the main stem. These responses are followed by death of the plant and disintegration of its tissues. Succulent plants that are susceptible to treatment with the acid often exhibit distorted growth for a period of 2-3 weeks following treatment, and they eventually die and are then readily digested by soil microorganisms.

There are marked differences in sensitivity between closely related plants when treated with 2,4-dichlorophenoxyacetic acid. For instance, *Plantago lanceolata* is extremely sensitive to treatment, while *P. major* is much less so. Bluegrass and crabgrass were relatively

insensitive, while certain closely cut bentgrasses were relatively sensitive. Little is known concerning the biological effects of growth-regulating chemicals when applied to soil in varying amounts. Accordingly, caution should be exercised in treating lawn or pasture areas until further information has been obtained as to the relative sensitivity of various kinds of plants and the effects of the compounds when present in the soil.

Summary

1. 2,4-Dichlorophenoxyacetic acid was effective as a differential herbicide when applied as an aqueous spray in concentrations of from 250 to 1000 p.p.m. or more.

2. Plants killed by these sprays were dandelion, narrow-leaf plantain, Dutch white clover, chickweed, pigweed, wood-sorrel, knotweed, broad-leaf dock, bindweed, and shiny pennywort. Other plants, such as broad-leaf plantain, sheep sorrel, daisy, yarrow, and various species of *Rubus*, were relatively insensitive to the acid.

3. Two applications of either 500 or 1000 p.p.m. concentration of the acid in aqueous solution were made to well-established Kentucky bluegrass sod without apparent injury to the grass. In addition, Kentucky bluegrass seed planted under a light top dressing of soil, which was then sprayed with the acid concentrations, germinated and readily became established in these lawn areas.

4. It was possible to obtain 95% control of dandelion and narrow-leaf plantain by a single spray application of a solution containing 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid or with two

applications at 500 p.p.m. concentration.

5. Caution should be exercised in the use of sprays containing the acid until more information is obtained concerning the effects of its presence in the soil.

Appreciation is expressed for assistance and helpful suggestions from L. W. KEPHART.

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SELECTIVE HERBICIDAL ACTION OF MIDSUMMER AND FALL APPLICATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID¹

C. L. HAMNER AND H. B. TUKEY

Introduction

In a previous paper (7), attention was called to the herbicidal action of 2,4-dichlorophenoxyacetic acid on bindweed, following suggestions by KRAUS (9) and MITCHELL and HAMNER (10). Mention was made of its effect upon several other species of plants, and suggestions were made for its use as a herbicide. The present paper discusses additional results when this acid is applied as a water spray on bindweed in cultivated areas; on sow thistle in a garden plot; on mixtures of common weeds in the field and in a bluegrass lawn; and on several woody plants. It also presents results with the aerosol method of application (5) and with the residual effect in the soil as evidenced by seed germination on treated areas.

¹ Journal Paper no. 605 of the New York State Agricultural Experiment Station.

Some of the results are fragmentary but seem worth presenting at this time.

MATERIAL AND METHODS.—All trials were conducted in the immediate vicinity of Geneva, New York, between July 14 and October 14, 1944. The soil of the area is characteristically heavy, has a pH of 6.5, and is provided with a fairly uniform moisture supply. The region is adapted to a wide range of horticultural and agronomic crops.

The season of 1944 was one of generally favorable growing conditions, with the possible exception of a period of drought and higher-than-normal temperatures during July. Rainfall and temperatures are given in table 1. Light frosts occurred on September 30 and October 16 and 17; the first killing frost was on October 20.

All trials were with 2,4-dichlorophenoxyacetic acid, secured from the

American Chemical Paint Company. Unless otherwise noted, it was applied in a water spray with a 5-gallon knapsack sprayer at a concentration of 1000 p.p.m., after first being dissolved in Carbowax 1500 at the rate of 1 part of the acid to 5 parts of Carbowax 1500, as described by MITCHELL and HAMNER (10).

TABLE 1

RAINFALL, MEAN MAXIMUM, MEAN MINIMUM, AND MEAN TEMPERATURES FROM JUNE TO NOVEMBER, 1944, AT GENEVA, NEW YORK

	June	July	Aug.	Sept.	Oct.
Rainfall (inches)...	3.96	1.72	2.37	2.43	1.75
Temperature (°F.)					
Mean maximum...	80.0	86.0	87.0	76.0	64.1
Mean minimum...	57.2	59.8	60.9	52.5	39.6
Mean.....	69.0	73.2	73.9	64.2	51.9

I. Results with application as a water spray

BINDWEED

Bindweed (*Convolvulus arvensis* L.) is one of the most noxious weeds in the United States (11). It reproduces freely from seeds and also very rapidly from underground roots and rhizomes. Roots may penetrate to depths of 10 feet (1, 3, 8), although the main horizontal roots are usually just above the watertable (8), and rhizomes may arise 30-59 inches below the surface of the soil (1). To test the effect of 2,4-dichlorophenoxyacetic acid on this weed, the following experiments were carried out.

EXPERIMENT 1.—In the preliminary report on the action of 2,4-dichlorophenoxyacetic acid on bindweed (7), the chemical was applied on July 14 to two 100-foot rows of apple nursery stock in an area so infested with this weed that it was felt it might have to be abandoned. The temperatures for several days both preceding and following the application

were approximately 80°-85° F. by day and 55°-60° by night. No rain fell for several days, either before or after application, and general field conditions were dry. Ten days after treatment, the above-ground parts of the plants were spongy, water-soaked, and enlarged to twice the diameter of similar parts from unsprayed plants. They soon decayed, at least to a distance of 14 inches below the surface.

Portions of roots and rhizomes dug from both treated and untreated plants were placed in a propagating frame to test their ability to regenerate. The portions from untreated plants made new growth (fig. 1), while those from treated plants failed to regenerate and died.

Following this response, nursery rows in an area of about 2½ acres were sprayed on July 31. One 600-foot row was left as a control. The area between the rows, which could be frequently cultivated, was not sprayed. The temperature was about 89° and the atmosphere dry. Within a few hours after the spray was applied, the field was disced and the soil thrown up to the rows of nursery stock so that it covered the bindweed with about 3 inches of moist soil. Ten days after treatment, the leafy parts of the bindweed were dead and the portions which are normally underground were decaying. The plants in the untreated areas between the rows showed many fresh shoots arising through the layer of soil which had covered them.

On November 9, when the soil was plowed away from the treated rows, no shoots or roots of bindweed were found to the depth of the plow; they had decayed and disintegrated. On the other hand, in the untreated row abundant live shoots and roots were uncovered in plowing, and vines were twined about the nursery stock.

EXPERIMENT 2.—On August 3, in order to test the method of entry of the chemical into the plant, the tips of individual plants of bindweed in the field were placed in vials containing 20 cc. of spray solution. Ten plants were used, three growing points or tips of each

EXPERIMENT 3.—On August 21, an area of about 2000 square feet containing a dense patch of bindweed was sprayed, but in this instance the solution was applied warm (110° F.). The area had been under frequent cultivation and the only weed growing was

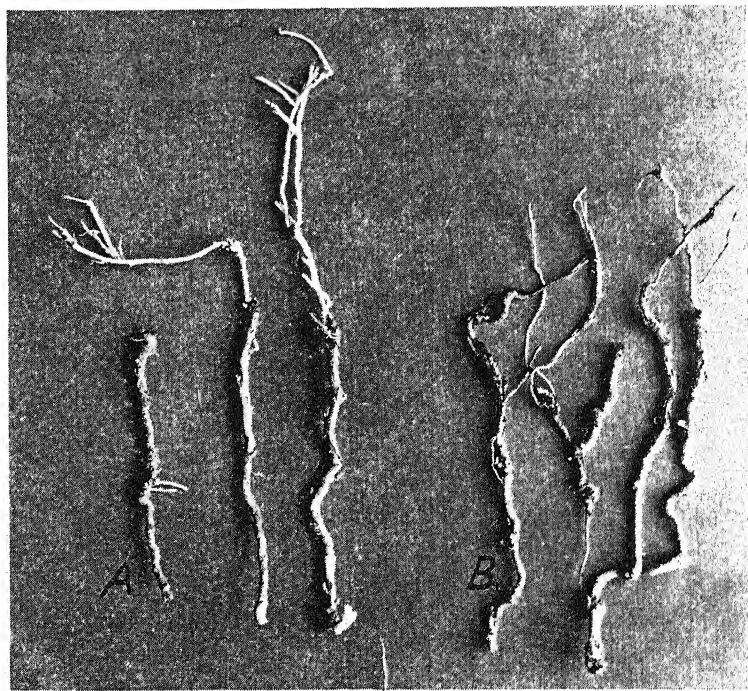


FIG. 1.—Effect of foliage spray on regeneration of roots and rhizomes of bindweed: *A*, untreated; new shoots developing. *B*, treated; proliferation, splitting, and decay.

plant being placed in each of the ten vials. For comparison, about 3 inches of the tips of ten other plants were covered to protect them from spray and the uncovered portions of the plants then sprayed with the same material. The responses were similar from both methods of application. Ten days after application all above-ground parts were dead, and below-ground parts were proliferated and split as previously described.

bindweed. It was in an active state of growth, and many young shoots had only recently emerged from the ground. The effect of treatment was pronounced. The tops were completely and uniformly killed, all at about the same time, whereas in other treatments not employing warm water some of the plants lingered a day or two after others were dead.

EXPERIMENT 4.—On September 9, on a relatively cool day (55° F.), about fifty bindweed plants were treated in a gar-

den plot. The air temperatures following the treatment were lower than those following the preceding treatments, with several cool nights (45° – 55° F.). The response was much slower, and complete killing of tops occurred only after 3 or 4 weeks.

SOW THISTLE

Sow thistle (*Sonchus arvensis* L.) is also a difficult weed (11) which regenerates rapidly from root pieces. To test the effect of 2,4-dichlorophenoxyacetic acid, a garden area was selected which was heavily infested. The garden had been hoed and cultivated during the early summer, so that the plants had been cut about 1 inch below the crown. Many root pieces had been severed and had apparently regenerated into a vigorous and persistent mass of plants. Individual plants were 12 inches in diameter, with leaves 10 inches in length.

The plot was divided into two sections, each consisting of about 200 square feet. On September 9, one section was given a very thorough foliage spray; the other left untreated. The weather was cool (about 55° – 60° F.) and rain had fallen for 2 days. After treatment the temperatures were 55° F. at night and 70° – 80° during the day.

Within 24 hours of treatment, the plants appeared lighter in color and lacked turgidity. Within 2 days the younger leaves showed severe twisting and curling. After 10 days the treated plants were still lighter green than the controls, and the outer row of leaves was flat on the ground, with a decidedly wilted appearance. The central group of leaves showed extreme curvature. The midribs near the base were enlarged and ribbon-like, and the bases were much enlarged and flattened, resembling celery stalks. The surface area occupied by in-

dividual treated plants was reduced by 60%.

The roots of the treated plants were greatly enlarged. An increase of at least 50% in diameter was common, and in some instances it was 300%. The roots were soft and spongy, showing much splitting and disintegration of the outer parts.

On September 25, 2 weeks after treatment, the untreated plants were completely dead, both tops and roots.

MIXTURE OF COMMON WEEDS IN FIELD

Applications were made variously to five areas on which were growing a range of weed plants common to the region, all of which are more or less of an economic problem. Most of them were at or near the flowering stage. Responses were much the same for all treatments, with a few exceptions that will be noted, so that only typically representative responses need be given:

1. Uncultivated orchard; mixture of twenty-two grasses and common weeds; 25×25 feet. Treated July 25; temperature 70° F., slightly cloudy, slight breeze blowing.

2. General farm land, cultivated until June 1; mixture of twenty-four grasses and common weeds, with germinating sweet clover; 24×36 feet. Treated August 21; temperature 75° F., sunny.

3. Headland of fertile truck farm; dense mat of purslane and pigweed, with scattering of seven other common weeds; 10×6 feet. Treated August 21; temperature 75° F., sunny.

4. Edging of stone driveway; mat of chickweed, dandelion, plantain, and grasses; 25×4 feet. Treated August 26; temperature 70° F., clear, sunny; light wind.

5. Rows of cultivated nursery trees; scattering of pigweed and purslane;

several hundred feet. Treated July 14, 80°–85° F., dry; and July 31, 89° F., dry.

In general, the greatest responses under these treatments were in those areas and under those conditions most favorable to plant growth. That is, the least effect was noted in the uncultivated orchard where plants were in a less active state of growth and the greatest in cultivated areas of good fertility. Further, responses were least during hot, dry conditions of July and with the more mature plants. At such times and under such conditions only the more active and less mature parts of the plants were affected—as tips, flowers, and below-ground meristematic parts. Later, when growing conditions were more favorable, as in late August, the responses were much more intense and affected a greater portion of the plant.

The responses of individual species, however, were characteristic:

Agropyron repens Beauv. (quack-grass); more than 100 plants.—No visible effect.

Amaranthus retroflexus L. (pigweed); fifty plants.—Within 3 hours main stem bent at 75–90 degree angle 6 inches from tip, becoming stiff; curvatures in petioles and leaves; at 1 day bending and stiffening of main stem progressing downward to within 2 inches of base on some plants, petioles stiff and recurved; at 4 days plants chlorotic, dry, woody, flower bracts brownish, flowers arrested, stems showing enlargement and splitting; at 29 days 50% of plants dead, remainder with a few tip leaves still functioning, rotting and blackening pronounced near split portions of stems (fig. 2).

Ambrosia artemisiifolia L. (ragweed); twenty plants.—Within 3 hours racemes showing severe curvature and becoming pendant (fig. 3); at 2 days all terminal growing points arrested; at 4 days plants

becoming progressively dry and brownish; at 29 days all plants brown and dead.

Asclepias syriaca L. (milkweed); ten plants.—Within 1 day second and third pairs of large leaves near apex of plant folded upward against main stem; at 2 days third and fourth pairs of large leaves folded upward, main stem bent to horizontal, bending progressing downward; at 9 days splitting, bending, and breaking of stems of four of ten plants just above ground, latex no longer present in plants examined; at 29 days unbroken plants recovering (fig. 4).

Avena fatua L. (wild oats); ten plants.—No visible effect.

Chenopodium album L. (lambs-quarters); thirty plants.—Within 1 day plants wilted in appearance, stems recurved at 2 inches from tip; at 2 days flowers and terminal growing points arrested, minor curvatures of stems, plants becoming stiff and woody; at 29 days stems split in many places, plants very rigid and woody, all dead.

Digitaria ischaemum Schreb. (small crab-grass); more than thirty plants.—Suggestion of swelling at nodes but no striking response.

Digitaria sanguinalis Scop. (large crab-grass); more than thirty plants.—Suggestion of swelling at nodes but no striking response.

Echinochloa crus-gallia Beauv. (barnyard-grass); ten plants.—No visible effect.

Eleusine indica Gaertn. (goose-grass); ten plants.—No visible effect.

Lycopersicum esculentum Mill. (tomato); two plants.—Within 1 day leaves folding upward along midrib, leaf stems curving downward, base of petioles enlarged; at 7 days leaves chlorotic, much enlargement of nodes, stems, and bases of petioles, terminal growing points arrested; at 29 days nodes greatly en-

larged, typical formative effects, root primordia on stems, leaves becoming brown, leaf area reduced 90% (fig. 2).

Malva rotundifolia L. (round-leaved mallow); twenty-five plants.—Within 3 hours leaves curved downward; at 1 day stems recurved at 3 inches from tips; at 2 days leaves becoming chlorotic,

land bare of the clover in contrast with adjacent field with abundant stand.

Plantago lanceolata L. (buckhorn plantain); three hundred plants.—Within 3 hours strong curvature of younger leaves; at 2 days leaves slightly chlorotic, roots with water-soaked appearance, discolored (fig. 5); at 10 days outer older

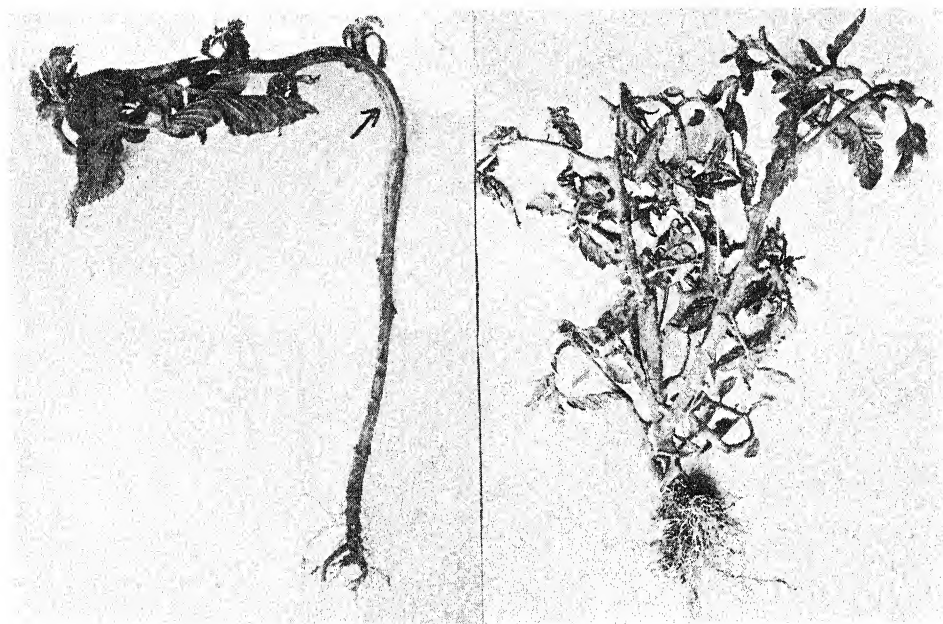


FIG. 2.—Effect of foliage spray on pigweed and tomato: Left, pigweed showing severe curvature and splitting of stem (arrow). Right, tomato showing enlarged stems, root primordia, swellings at nodes, curvatures, and arrested development.

flowers and terminal growing points arrested; at 13 days stems and parts of roots split, some callous formation in wounds, plants very stiff and woody, fifteen plants dead (fig. 3); at 29 days all plants dead and disintegrated in tops and roots.

Melilotus alba Desr. (white sweet clover); several thousand seedlings emerging 3–7 days after treatment to soil.—Severe spiral twisting of plants and downward folding of leaves as plants emerged, complete killing; at 29 days

leaves flattened to ground, younger flower stalks white, elongate and curled, old flower stalks brown, central younger leaves curled, bases of leaves whitish, thickened, crinkled, ribbon-like, base of leaves and upper root sections rotting, complete absence of new flower stalks; at 30 days all plants dead, tops and roots disorganized and disintegrated.

Plantago major L. (broad-leaved plantain); fifty plants.—Within 1 day epinasty of leaves and petioles, young flower spikes recurved; at 2 days base of

leaves white, elongate, flat, ribbon-like; at 4 days mature flower spikes brown and drying out, young spikes etiolated; at 29 days 50% of plants dead, others

Polygonum pennsylvanicum L. (Pennsylvania smartweed); ten plants.—Within 1 day plants severely flattened to ground; at 2 days flowers checked in

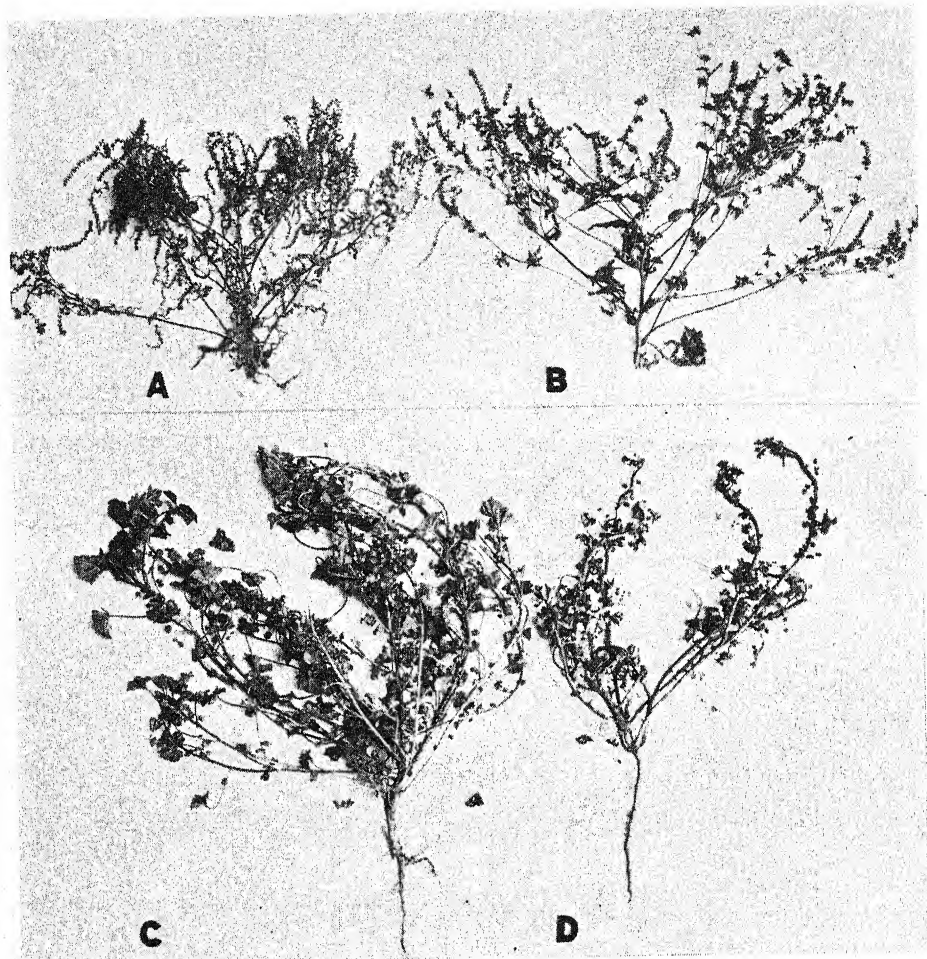


FIG. 3.—Effect of foliage spray on ragweed and round-leaved mallow: A, ragweed, sprayed, showing severe curvature of racemes. B, ragweed, untreated. C, round-leaved mallow, untreated. D, round-leaved mallow, sprayed, showing severe check in growth and flower development.

alive but with many torn leaves, dead flower stalks, and no new growth of either flower stalks or vegetative portions.

Poa pratensis L. (Kentucky bluegrass); more than 1000 plants.—Within 10 days darker green color, no visible injury.

development; at 29 days all plants dead.

Portulaca oleracea L. (purslane); two hundred plants.—Within 3 hours leaves becoming recurved; at 1 day tendency of plants to cling to ground, stems brittle; at 2 days foliage and stems chlorotic; at 7 days some leaves abscising, stems

very brittle; at 29 days most plants still alive, stems cracking and splitting, decay entering wounds, stems easily broken, almost all axillary growth on main stem inhibited, a few leaves still alive near tips of stems, foliage reduced 90%, much curvature of stems, seed production reduced to less than 1% that of untreated plants.

Setaria lutescens Hub. (yellow foxtail); twenty-five plants.—No visible effect.

Setaria viridis Beauv. (green foxtail); twenty-five plants.—No visible effect.

Stellaria media Cyrill. (chickweed); more than 100 plants.—Within 5 days curvature of stems and severe browning of leaves, flowers arrested in development; at 13 days 90% of plants dead.

Taraxacum officinale Weber. (dandelion); three hundred plants.—Within 3 hours strong curvature of center leaves; at 1 day center leaves twisted 180°; at 2 days base of leaves whitish, elongate, enlarged; at 10 days leaves chlorotic, flaccid at base, complete absence of new flower stalks, old flower stalks elongate, chlorotic, and twisted (fig. 5); at 13 days all plants dead; at 30 days all roots disorganized and disintegrated.

Trifolium pratense L. (red clover); twenty-five plants.—Within 13 days browning of leaves touched by spray, plants subsequently recovered.

MIXTURE OF COMMON WEEDS IN BLUEGRASS LAWN

The selective response observed in treatments of field mixtures of weeds made on July 25, in which plants of broad-leaved plantain, buckhorn plantain, and dandelion were killed whereas several grasses—including yellow foxtail, green foxtail, quack-grass, and Kentucky bluegrass—were not affected, suggested applications to bluegrass lawns infested with weeds.

EXPERIMENT 1.—On August 21, applications were made to a bluegrass lawn infested with dandelion and broad-leaved and buckhorn plantain, applying the material as a warm water spray (110° F.) directed at forty to fifty individual plants here and there on the lawn.

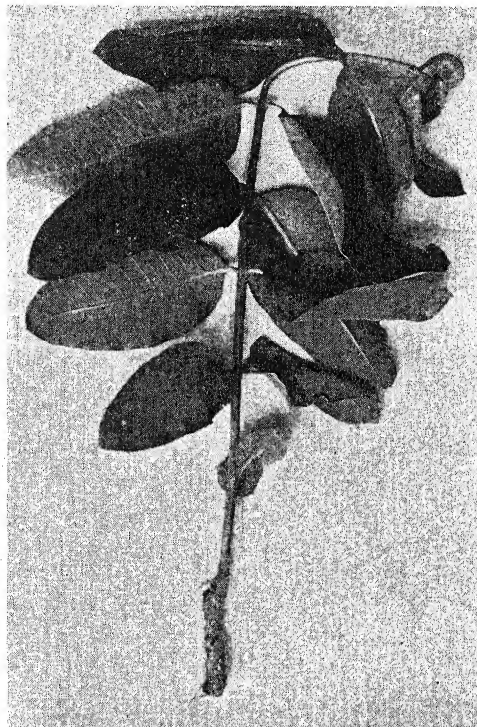


FIG. 4.—Effect of foliage sprays on milkweed, showing hyponasty and bending of stem.

Treated plants of dandelion and buckhorn plantain responded typically within a few hours, and all were dead within 10 days. Plants of broad-leaved plantain were less affected, and many were not killed although in a weakened condition. There was no visible injury to the grass.

EXPERIMENT 2.—On August 26, a carefully measured area 10 X 10 feet was selected in the center of a bluegrass lawn. The area contained 158 plants of buckhorn plantain, forty-five of dande-

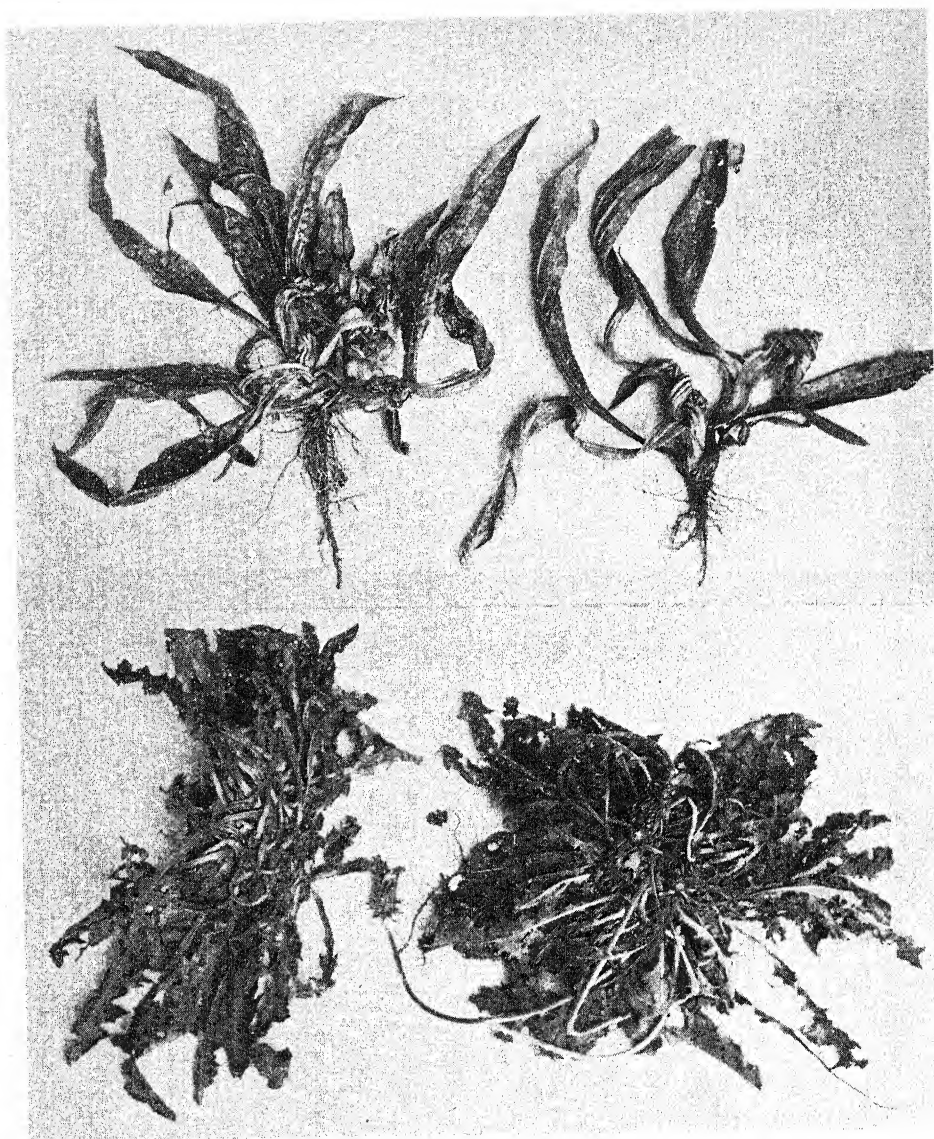


FIG. 5.—Effect of foliage sprays on buckhorn plantain and dandelion, showing curvature, twisting, elongation of leaf bases, and arrest in flower development. Above, buckhorn plantain; below, dandelion.

lion, nine of round-leaved mallow (*Malva rotundifolia* L.), and a scattering of white clover (*Trifolium repens* L.). The spray was applied generously at a temperature of about 100° F. Six quarts of spray were used for the area. The weather was clear and sunny, and the air temperature was 70° F.

Within 2 days the plantain, dandelion, and mallow showed typical curvatures and chlorosis, and the main roots appeared water-soaked and discolored. The leaves of the white clover plants were curved downward. Within 13 days all plants of dandelion and plantain were dry and either completely dead or dying, the stems of mallow had proliferated and split, and the growth of white clover was severely checked. By September 25, 30 days after treatment, all plantain, dandelion, and mallow plants were dead, the tops being dried up and the roots disorganized. By October 17 not a single plant of these species remained alive, the only traces of any of them being a few brown vascular strands and fibers. Most of the white clover was dead, and that which was not entirely so was killed back to the main stolons and making feeble new growth, in contrast to the vigorous growth of clover in immediately adjacent unsprayed areas.

The bluegrass showed no evidences of injury that could be detected at any time. On the contrary, within 10 days of treatment the square of treated lawn was darker green in color and more vigorous than the rest of the lawn. On October 17, 52 days after treatment, the treated area was entirely free of dominant plant material other than grass and was sharply defined from the surrounding lighter green and less vigorous lawn area infested with plantain, dandelion, white clover, and mallow.

WOODY PLANTS

A few woody plants were sprayed at a concentration of 1000 p.p.m. during late July and early August, when they were relatively inactive. The results are fragmentary but are presented because of the contrast with herbaceous material. They do not preclude the possibility that higher concentrations and different times and methods of application might not produce different effects.

Malus domestica Borkh. (common apple); ten plants.—Minor curvature of tips and slight chlorosis of leaves near tips.

Rhus toxicodendron L. (poison ivy); 200 plants.—Within 1 day leaves drooping, new growth chlorotic; at 7 days leaves becoming more chlorotic; at 14 days slight recovery; at 36 days climbing plants defoliated; at 66 days all plants prematurely defoliated; at 76 days 50% of above-ground parts killed.

Rubus strigosus Michx. (red raspberry); twenty plants.—Curvature 3-6 inches back from tips, petioles re-curved, leaves folded upward along midrib, growth of tips arrested, stiff.

Rubus flagellaris Willd. (dewberry); twenty plants.—Within 1 day curvature 3-6 inches back from tips, leaves dry and brittle; at 75 days tips which were formerly curved killed back 4 inches.

Vitis vulpina L. (frost grape); ten plants.—Within 7 days curvature and browning of tips, resulting in killing of terminals back 4 inches.

II. Results with application as an aerosol

It was suggested in a previous paper (5) that perhaps the herbicidal effectiveness of 2,4-dichlorophenoxyacetic acid could be increased by applying it as an aerosol. This method has been used re-

cently for growth-regulating substances and is more fully described elsewhere (6). It is more nearly comparable to fumigation than to spraying.

An aerosol was prepared with 2% dichlorophenoxyacetic acid in 10% motor oil SAE 30 and 88% dimethyl ether. On September 9 it was applied from a 1-quart dispensing cylinder to two rows of apple nursery stock infested with bindweed and occasional plants of pigweed and purslane. The nozzle of the cylinder was directed downward at arm length and kept open while the operator walked rapidly down the row. The ground area covered was about 60 square yards, and half a pound of aerosol was used. No attempt was made to conserve material, so that no accurate data were obtained as to just how small an amount might have been equally effective. The temperature preceding the application was 55° F. Because of a brisk wind blowing, the aerosol drifted considerably.

In another area the material was applied horizontally about 2 feet from the ground with the small nozzle removed and discharged in a broad fanlike sweep 10 feet wide and 30 feet long.

Sixteen hours after treatment the plants of bindweed, purslane, and pigweed under both methods of application showed typical yet violent curvature, together with slight chlorosis. Plants were affected 30 feet to one side of the treated areas and beyond the point where any visible drifting of the material had occurred. Aerosol treatment can be more or less confined by the type of nozzle used, and no attempt was made in these treatments for spot application. Nevertheless, the effect of the material over such a broad area, and in what must have been very small concentrations, is of interest.

Five days after treatment many of the

plants were dead, and 10 days after treatment all were dead. The plants treated by downward application at arm length were especially severely affected, probably owing to their receiving a higher concentration at close range than was the case with plants treated by a broad horizontal sweep.

The aerosol treatment was the most rapid and most effective treatment of any tested. The action is all the more striking since the air temperatures (55° F.) were lower than when the most effective liquid spray treatments were made (65°–90° F.) and in view of the fact that liquid-spray treatments at low temperatures (55° F.) were more slowly effective.

III. Residual effect in soil

To determine the residual effect of 2,4-dichlorophenoxyacetic acid in the soil following applications as a herbicide, two treated areas were seeded to several cereal, lawn, and pasture plants.

EXPERIMENT 1.—One of the areas was the field mixture of common weeds which had been treated on August 21 with a light spraying at 1000 p.p.m. Vegetation was sparse at the time of treatment, and there is every likelihood that some material was applied directly to the soil surface, in addition to that which may have run off the foliage of sprayed plants. It was this area which had been seeded to white sweet clover just prior to the treatment and in which all young seedlings were killed as they emerged.

Plots 2 feet square were planted with cereals, grasses, and pasture plants on October 18 (58 days after treatment) under conditions favorable to germination. For comparison, similar plots were seeded on an untreated area immediately adjacent. By November 9, 22 days

after seeding, an excellent stand was secured of all species. No evidences of curvature or distortion were observed.

EXPERIMENT 2.—The second area was the lawn plot (10 × 10 feet) which had been sprayed with 6 quarts of material on August 25, and on which the killing of plantain, dandelion, and mallow had left bare spots. On October 18 (54 days after treatment) the bare spots were lightly seeded with Kentucky bluegrass. These, too, germinated and were not visibly affected.

Discussion

Many herbicides depend for their effectiveness on their caustic action, whereas the effectiveness of 2,4-dichlorophenoxyacetic acid depends on its conduction throughout the plant, the growth responses induced, and the destruction of specific tissue systems. Not only is the material selective for different species of plants, but it is also selective in the type of response which it brings about in different plants and different parts affected. Thus, the flowers and terminal growing points of above-ground parts of some plants may be arrested (as bindweed), or killed (as dewberry and grape). Some may become chlorotic (as poison ivy). Some may show epinasty (as purslane), and others may show hyponasty (as milkweed). The progressive curvature downward from the tips and the stiffening, dying, and splitting of above-ground stems is typical of pigweed, while the wilted appearance of the foliage of sow thistle and bindweed, and the proliferation, splitting, discoloration, and water-soaked appearance of the below-ground points are also typical.

The method of killing is of special significance when applied to such plants as bindweed and sow thistle, which regen-

erate not only from seed but also from shoots arising from underground stems and roots. BEAL (2) has shown that "pronounced morphological responses are often induced or incited at considerable distance from the point of application," and he has designated this response as "telemorphic." This is apparently the case with bindweed. How far the material travels may play a large part in its effectiveness as a herbicide.

2,4-Dichlorophenoxyacetic acid is especially effective against plants when in the meristematic condition. Actively growing bindweed and sow thistle are highly sensitive to this acid; and when thus stimulated, the increased activity must result in depletion of reserves to some degree. This depletion has been shown to be an important consideration in weed control (1, 4).

The apparent lack of response of the grasses at the time and concentrations used suggests the possibility of controlling undesired plants in lawns and turfs. There is also the suggestion from the re-seeding treatments that light surface applications to cultivated land sometime prior to seeding might destroy some undesired weeds in young stages and yet not affect germination of the crop sown at a later date. A detailed study of the mechanism of action, responses to different concentrations, and effect of applications at different times and by different methods should yield profitable results.

Summary

1. Experiments were conducted in the immediate vicinity of Geneva, New York, between July 14 and October 14, 1944, using 2,4-dichlorophenoxyacetic acid as a herbicide applied as a water spray at a concentration of 1000 p.p.m. in Carbowax 1500 at the rate of one part acid to five parts Carbowax.

2. Applications to bindweed in rows of nursery stock on July 14 and July 31 resulted in drying out and killing of above-ground parts. Below-ground parts proliferated, became spongy and water-soaked, and decayed to a depth of at least 14 inches. Applied in warm water (110° F.), killing was more uniform and more rapid. In relatively cool weather (45°-75° F.) the response was much slower, and complete killing occurred only after 3-4 weeks. Bindweed was killed by immersing the tips in vials of the solution, and they were also killed when the foliage was sprayed exclusive of the tips.

3. Applications to sow thistle growing in a garden plot resulted in lighter green color and wilted appearance within 24 hours. The bases of the leaves became much enlarged and flattened, and the roots increased 50-300% in diameter and became soft and spongy. All plants were dead within 2 weeks.

4. Applications were made to field mixtures of common weeds. There was no visible effect upon quack-grass, Kentucky bluegrass, yellow foxtail, green foxtail, wild oats, large crab-grass, small crab-grass, barnyard grass, and goose-grass. Bindweed, narrow-leaved plantain, dandelion, round-leaved mallow, lambs-quarters, and ragweed were killed following varying formative responses. Pigweed, milkweed, tomato, broad-leaved plantain, Pennsylvania smartweed, purslane, chickweed, and red clover showed varying responses, including epinastic curvatures, splitting of stems, swelling of stems and roots, browning of leaves, stems, and roots, chlorosis of leaves and stems, elongation, chlorosis

and enlargement of petioles, and killing of some plants.

5. Germinating seedlings of white sweet clover emerging 3-7 days after the soil surface had been sprayed were completely killed.

6. Applications were made to a bluegrass lawn infested with dandelion, buckhorn plantain, round-leaved mallow, and white clover. All plants of dandelion, plantain, and mallow were dead and disintegrated within 30 days; most of the white clover was dead, and that which was not dead was killed back to the main stolons; and the bluegrass became dark green in color but otherwise not visibly affected.

7. A few woody plants were treated. The apple showed minor curvature and mild chlorosis near the tips; poison ivy showed chlorosis and arrested development and 50% killing of above-ground parts; red raspberry, formative effects 3-6 inches back from the tips; and dewberry and grape showed formative effects and killing 4 inches back from the tips.

8. Applications by the aerosol method, using 2% 2,4-dichlorophenoxyacetic acid in 10% motor oil SAE 30 and 88% dimethyl ether, proved effective against bindweed, pigweed, and purslane.

9. The residual effect of the acid in the soil after application as a herbicide was tested. Twelve species of cereal, lawn, and pasture plants were sown on an area which had been sprayed 2 months earlier. The seeds germinated and the young plants showed no curvatures or formative effects.

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CURRENT LITERATURE

Plants and Vitamins. By W. H. SCHOPFER. Translated by N. L. NOECKER. Waltham, Mass.: Chronica Botanica Co., 1943. Pp. 293. Illustrated. \$4.75.

A great deal of the attention of biologists has been and is absorbed in the study of growth, and with good reason, for it is one of the most significant characteristics of living matter. When it is found, therefore, that the growth of any organism depends upon the presence of a minute quantity of a single substance, the discovery naturally affects the thinking of all those who are interested in this most important process. SCHOPFER's demonstration of the essential nature of thiamine for the development of the bread mold was a discovery of that type, and its results have profoundly influenced many researches. As the ripples from a stone dropped in a pool spread to a distant shore, so the discovery that some plants suffer from vitamin deficiencies has had far reaching effects.

SCHOPFER's discovery is interesting in another respect. It shows how the investigation of a phenomenon which on the surface appears trivial may have very considerable ultimate results. SCHOPFER found the essential nature of thiamine for *Phycomyces* by searching for an explanation for the failure of

this fungus to grow when one sample of maltose was used as a source of carbon and its ability to flourish when another sample of maltose was supplied. The answer, as he eventually learned, was the presence of thiamine (or its intermediates) in the one sample and its absence in the other. These preliminary remarks will explain in part why SCHOPFER should have written this book and why his text is of interest to botanists and to all others concerned with the problem of growth.

The book contains twenty-four chapters, with a conclusion, author index, general index, and illustrations. It is divided into three parts as follows. Part I: Synthesis of vitamins in plants; Auxotrophic plants; Research methods. Part II: Vitamins in relation to plants unable to synthesize them; Growth factors for microorganisms. Part III: General problems involving vitamins. Each chapter is followed by a bibliography. The text is clearly written, and the author has not hesitated to include chemical formulae and chemical discussion when necessary to elucidate the topic under consideration.

Much has been added to the field since the text was completed, but it stands as a source book which should be available to everyone who works with plants, both phanerogams and cryptogams.—W. J. ROBBINS.

Thomas Jefferson and the Scientific Trends of His Time. By CHARLES A. BROWNE. Waltham, Mass.: Chronica Botanica Co., 1944. Pp. 423. \$1.25.

This is a brief presentation of some of the salient facts concerning THOMAS JEFFERSON'S versatility, including some of his ventures and speculations in the fields of science. His statement that "it is always better to have no ideas than false ones; to believe nothing rather than to believe what is wrong," seems to be part of the current preachments of present-day educators as well.—E. J. KRAUS.

Aquatic Plants of the United States. By WALTER C. MUENSCHER. Ithaca, N. Y.: Comstock Publishing Co., 1944. Pp. 374. Illustrated. \$5.00.

This contribution helps greatly toward filling the gap in available information on aquatic plants. The material, which covers the entire United States, is presented in a manner so clear that interested laymen having a general knowledge of botanical terminology and trained botanists alike will find it most useful.

Diagnostic keys to families, genera, and species, simple clean-cut critical drawings, and distributional maps all aid in making identification more simple and direct.—E. J. KRAUS.

The Naturalist's Lexicon. By ROBERT S. WOODS. Pasadena, Calif.: Abbey Garden Press, 1944. Pp. 282. \$2.75.

To biologists interested in the English equivalents of some technical names and phrases, or in inventing new technical terms, this brief work should prove useful. Most of the space of the book is devoted to a Classical-English lexicon, followed by a much briefer English-Classical supplement, in which may be found a limited list of nouns, adjectives, verbs, and prefixes relating to both animals and plants.—E. J. KRAUS.

The Aquatic Oomycetes of Wisconsin: Part I. By FREDERICK TAYLOR WOLF. Madison, Wis.: University of Wisconsin Press, 1944. Pp. 64. \$1.50.

This brief volume contains an assemblage of the results of the investigations of the several previous collectors' reports on the aquatic Oomycetes of Wisconsin, combined with the findings from several thousand collections of water and soil samples made by the author during a period of more than 2 years and cultured for the presence of aquatic fungi. Adequate descriptions of the forms, records of their collections, and keys for their identification have been included. Slightly more than fifty species are listed.—J. M. BEAL.

A Source Book of Agricultural Chemistry. By CHARLES A. BROWNE. Waltham, Mass.: Chronica Botanica Co.; New York City: G. E. Schert Co. \$5.00.

The author gives as his definition of agricultural chemistry, "that branch of applied science which deals with the chemical composition and mutual chemical relations of soils, fertilizers, crops, and farm animals in so far as they concern the production upon the farm of agricultural supplies," and states further that "The mutual relationships of crops to animals and of animals to crops constitute, however, such an important part of agricultural science that they must be considered in any comprehensive treatise of agricultural chemistry."

With this as a basis for selection of material, a history of the subject from the early Greek nature philosophers (640-430 B.C.) through most of the experimentally productive activity of LIEBIG—terminating with 1852—is presented. Students of plant physiology will find much that is familiar, since most of the subject matter deals with plants in relation to their environment. Illustrative material and quotations are presented to much greater length and with far more detail than is usually encountered in most botanical text-books now available. Comments by the author are to the point and often helpfully critical. The book is a substantial addition to the literature treating of botanical history.—E. J. KRAUS.

RELATIVE YIELDS OF INBRED LINES AND F_1
HYBRIDS OF TOMATO

LEROY POWERS

Introduction

The relative merits of inbred lines and F_1 hybrids for increasing yield of tomatoes is one of the most important problems confronting tomato breeders today. POWERS (13, 14), POWERS and LYON (16), and LYON (12) have published data on the F_1 hybrids, F_2 generations, first generation backcrosses to both parents, and the parents which showed that the use of F_1 hybrids for the commercial production of tomatoes has considerable promise. The desirability of using genetically diverse parents as a basis for producing the inbred lines to be used as parents for the F_1 hybrids was given special emphasis. The following crosses were used in establishing the inbred lines: Danmark \times Johannisfeuer, Johannisfeuer \times Bonny Best, Danmark \times Red Currant, Johannisfeuer \times Red Currant, Danmark \times Ponderosa, and Ponderosa \times Porter. By 1941 and 1942, some of the inbred lines were sufficiently close to homozygosity to justify further studies concerning the relative merits of these inbred lines in F_1 hybrid combinations. The literature dealing with the merits of F_1 hybrids derived from crossing existing commercial varieties and the problems involved in the production of first-generation hybrid tomato seed for commercial planting has been discussed by BARRONS and LUCAS (3) and BARRONS (2). The primary purpose of this study is to evaluate the F_1 hybrids derived from inbred lines produced from parents of wide genetic diversity.

Of particular importance in attaining this objective are: the partitioning of the different characters into their components; studying the range in variation of these characters; determining to what extent the desired expression of a character or combination of characters can be attained in the inbred lines and F_1 hybrids; and finally, determining the combining ability of the various inbred lines as regards the characters under consideration. Heterosis and dominance are important in determining the combining ability or extent of expression of a character in the F_1 hybrids. Some of the genetic factors regulating the ease or difficulty with which the genes differentiating the various characters can be recombined in individual F_1 hybrid populations and inbred lines are the number of gene pairs differentiating the character or characters under consideration, the linkage relations of these genes, and pleiotropy.

The climatic conditions at Cheyenne, Wyoming, are those typical of the higher Great Plains region. The elevation above sea level of the Cheyenne Horticultural Field Station is 6300 feet. The average time between the last killing frost in the spring and the first in the fall is 141 days. The average annual precipitation is 15.82 inches. However, additional moisture as needed was supplied by irrigation to the experimental plots from which the data for this study were taken. For these climatic data and further details concerning the climatic conditions existing at Cheyenne, see KINCER (11).

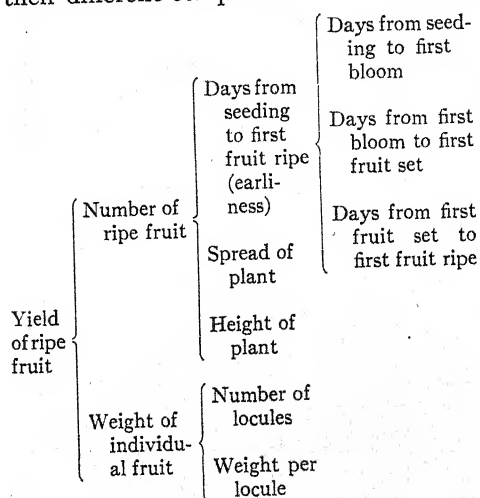
Material and methods

The material grown during 1941 consisted of the inbred lines Red Currant, Johannisfeuer, Danmark, their possible F_1 hybrids, and three inbred lines derived from the cross Johannisfeuer \times Red Currant. The material grown in 1942 consisted of ten inbred lines and all the different hybrids possible among them. Eight of the inbred lines were derived by continued selection of individual plants from the later generations of the hybrids previously reported on by POWERS (14), POWERS and LYON (16), and LYON (12); the other two were Bounty and Danmark, these being used because they were the most outstanding commercial varieties tested at the Cheyenne station, where the studies were conducted. This investigation is in reality a continuation and expansion of the studies reported in the preceding citations.

The experimental design consisted of ten randomized complete blocks, one plot of each variate being grown per block. Each plot was composed of twelve plants 4 feet apart within rows and the rows spaced 4 feet apart. Since the interest in this study is in the comparisons between the means of the F_1 hybrids and inbred lines, the plot instead of the plant was used as the unit for reducing the data. Then, for each parent and generation the standard errors were estimated for within the F_1 hybrids and inbred lines on the basis of the ten-plot means, these means having been derived from the individual plant data for the respective plots. In all tables the standard errors of the means are given. As in the previous work, cited above, a generalized error was not used; instead, a standard error was calculated for each variate on the basis of the ten-plot replicates of that variate. The data for weight of ripe fruit per plant, number of ripe fruit, and

weight of individual fruit were taken from two plants of each plot. These two plants were chosen at random by means of TIPPETT's tables (19). The data for all other characters were taken from all plants of each plot.

The method employed in studying the characters was to partition them into their different components:



Yield per plant is the weight in grams of the fruit that ripened during the growing season. Spread and height of plant are expressed in centimeters; height is the distance from the ground level at the base of the plant to the top, and spread is the diameter measured through the main stem as the center. Days was the unit employed for measuring earliness of maturity and its different components. The details of how the earliness of maturity measurements are taken are given in a previous article (16). Weight per fruit in grams was obtained by weighing the ripe fruit and dividing by the number that ripened. Number of locules was determined from transverse sections of the fruit. Weight per locule was obtained by dividing weight of fruit by number of locules. The results from the studies on the interrelations of weight per fruit,

number of locules, and weight per locule show that weight per locule (as determined) is a character of considerable importance in breeding larger-sized fruits. Data supporting this conclusion are given in the section on the relation between weight of individual fruit, number of locules per fruit, and weight per locule (p. 265).

Tables of means are given for all characters except number of days from seeding to first bloom, number of days from first bloom to first fruit set, and height of plant.

It should be noted that the variation of yield, weight of individual fruits, and number of days from seeding to first fruit ripe is completely accounted for by the multiple regression of these characters on their respective components; that is, R^2 multiplied by 100 should equal 100%, if the precise description of the concomitant variation of the quantities has been discovered and the appropriate transformation used in calculating the data. Hence, the closeness with which R^2 times 100 approaches the value 100% is a measure of the agreement between the assumed and the actual nature of the covariation of the variates; that is, if the proper scale of measurement (21, 5) has been used in determining the R^2 and r^2 values.

Also, it should be noted that r^2 times 100 is the percentage of the variation (either sums of squares, Sy^2 or Sx^2 , or variance, Vy or Vx) that can be accounted for by covariation; that is, by the regression of one character on the other. For definition of terminology, use of symbols, and methods of reducing the data, see SNEDECOR (17).

The method of grouping the means may be seen in table 1. The inbreds may be grouped into four main classes on the basis of their behavior in crosses: (a)

The first class is composed of 4101, 4102, and 4105; (b) the second class of 4103 and 4104; (c) the third class of 4106, 4107, and 4108; and (d) the fourth class of 4109 and 4110. In the tables the data for the inbreds and hybrids among inbreds within each group are blocked off by bold-faced lines, while interclass hybrids lie outside these lines. The interclass hybrids between the inbred lines are further delimited by broken lines.

The use of the terms heterosis and dominance throughout this paper is consistent with the information on these phenomena reported by POWERS (14, 15).

Experimental results

YIELD OF RIPE FRUIT PER PLANT

The average weights of ripe fruits per plant harvested during the growing season of 1942 are given in table 1. The grand means of the inbred lines and those of the hybrids are listed in the last row of this table. The F_1 hybrids are far superior to the inbred lines in yielding ability, producing 59% more ripe fruit by weight. That the average yield of any inbred line is surpassed by the grand mean of its respective hybrids can be seen by comparing the figure at the foot of each column with the figure in the same row of the last column. Only six of the forty-five hybrids failed to show heterosis.

Of the four classes depicted by the solid demarcation lines, no. 3 is the only one in which intraclass heterosis is questionable, indicating that these inbred lines of class 3 have inferior combining ability. That they lack superior combining ability with some other inbred lines as well is shown by the fact that five of the six hybrids not showing heterosis had these inbred lines as parents.

The outstanding hybrid combinations in regard to yield are 4109 \times 1410 and

4102 \times 4110. They yielded approximately three times as much as Danmark, the variety recommended for commercial production by BABB and KRAUS (1). Of the hybrids having larger-sized fruit, 4101 \times 4103 was the highest producer, yielding 1827 gm. of ripe fruit per plant as compared with 828 gm. for Danmark. These data indicate the striking increase in yield attainable by utilizing heterosis.

The inquiry should not end with the discovery of the high yielding combinations; rather, the origin of these inbred lines should be considered in relation to their ability to produce high yielding hybrids. The inbred lines fall into two groups as to parentage: 4101, 4102, 4105, and 4106 are of *Lycopersicon esculentum* Mill. and the other six have resulted from crosses involving *L. esculentum* and *L. pimpinellifolium* (Jusl.) Mill. (Red Currant). From the data of table 1 it can be seen that none of the F_1 hybrids involving inbred lines derived solely from *L. esculentum* possessed special yielding ability. All the high producing F_1 hybrids have at least one parent derived from crosses between *L. esculentum* and *L. pimpinellifolium*. Again these findings emphasize the importance of developing and selecting the inbred lines in such a way that they have as wide a diversity of genes initiating those substances and reactions responsible for the development of the character sought as it is possible to attain.

The outstanding inbred lines in F_1 hybrid combinations (table 1) are themselves the three highest yielders—4110, 4109, and 4103. Just how far the breeder can go in employing yield of inbred lines to discover those having exceptional combining ability cannot be deduced from these data. Extensive investigations on heterosis and dominance involving numerous and diverse breeding stocks are required before conclusions of

general application to combining ability can be drawn.

NUMBER OF RIPE FRUIT PER PLANT

The means for number of ripe fruit per plant are listed in table 2. The data show that the grand mean of the inbred lines and the grand mean of the F_1 hybrids are not greatly different and that the hybrids for the first eight inbred lines (4101 to 4108, inclusive) average a greater number of ripe fruit per plant than their respective inbred lines, whereas the reverse is true for similar comparisons involving 4109 and 4110. Obviously, the behavior of the hybrids is not the same, and therefore the data of this table must be considered in more detail.

The intraclass hybrids of classes 1, 2, and 4 show heterosis, whereas those of class 3 do not. Also, the interclass hybrids between classes 2 and 3 have a tendency toward a slight degree of heterosis. The interclass hybrids of 1 and 2 and of 1 and 3 show partial dominance of greater number of fruit per plant, the degree of expression approximating rather closely that of complete dominance. The interclass hybrids involving classes 2 and 4, and those involving classes 3 and 4, show partial dominance for greater number of fruit; but the degree of expression is only a little above that of no dominance. On the whole, the interclass hybrids involving classes 1 and 4 show partial dominance for fewer fruit per plant. Again, the degree of partial dominance is not marked, being not greatly different from no dominance. These data show that in the F_1 hybrids the expression of number of ripe fruit per plant ranges from partial dominance of fewer fruit to heterosis for more fruit per plant. This statement has added importance when one considers that the range in this material is from 2.9 to 183.2 ripe fruit per plant.

TABLE 1
MEANS FOR YIELD (IN GRAMS) OF RIPE FRUITS PER PLANT HARVESTED DURING GROWING SEASON*

Variety or inbred line	Bounty 4101	[(D×J)×D] S ₆ 4102	[(J×BB)×BB] S ₅ 4105	[(D×RC)×D] S ₅ 4103	[(D×RC)×D] S ₅ 4104	Danmark 4106	[(D×RC)×D] S ₆ 4107	[(D×RC)×D] S ₆ 4108	(J×RC) F ₉ 4109	(J×RC) F ₈ 4110	Grand means for hybrids
1. Bounty, 4101	513 ± 39	934 ± 182	675 ± 98	1827 ± 196	1216 ± 138	1036 ± 168	907 ± 131	964 ± 128	1653 ± 168	2310 ± 186	1280 ± 53
[(D×J)×D] S ₆ 4102		607 ± 86	882 ± 142	1367 ± 148	1165 ± 132	707 ± 118†	871 ± 127	934 ± 140	2059 ± 81	2428 ± 150	1267 ± 46
[(J×BB)×BB] S ₅ 4105			332 ± 64	1352 ± 138	890 ± 93	798 ± 79†	598 ± 83†	693 ± 75†	2004 ± 33	1831 ± 107†	1081 ± 33
2. [(D×RC)×D] S ₅ 4103				1060 ± 159	1459 ± 136	1433 ± 127	1551 ± 104	1270 ± 128	1842 ± 220	2266 ± 127	1597 ± 54
[(D×RC)×D] S ₅ 4104					808 ± 114	1186 ± 111	1195 ± 106	1208 ± 97	1537 ± 71	2201 ± 105	1340 ± 44
3. Denmark, 4106						828 ± 108	929 ± 169	860 ± 92	1969 ± 119	2144 ± 102	1236 ± 45
[(D×RC)×D] S ₆ 4107							801 ± 111	785 ± 138†	1852 ± 122	1042 ± 91	1181 ± 47
[(D×RC)×D] S ₆ 4108								857 ± 108	1921 ± 154	2882 ± 136	1192 ± 41
4. [(J×RC) F ₉ 4109									1364 ± 151	2876 ± 177	1908 ± 46
[(J×RC) F ₈ 4110										1868 ± 149	2231 ± 52
Grand mean yields of inbred lines and of hybrids, respectively.										904 ± 36	1437 ± 15

TABLE 2
MEANS FOR NUMBER OF RIPE FRUIT PER PLANT*

Variety or inbred line	Bounty 4101	[(D×J)×D] S ₆ 4102	[(J×BB)×BB] S ₅ 4105	[(D×RC)×D] S ₅ 4103	[(D×RC)×D] S ₅ 4104	Danmark 4106	[(D×RC)×D] S ₆ 4107	[(D×RC)×D] S ₆ 4108	(J×RC) F ₉ 4109	(J×RC) F ₈ 4110	Grand means for hybrids
Bounty, 4101	4.3 ± 0.32	8.2 ± 1.40	6.0 ± 1.01	20.5 ± 2.27	14.4 ± 1.99	10.5 ± 1.69	8.8 ± 1.32	8.9 ± 1.29	54.0 ± 5.26	55.9 ± 5.92	20.8 ± 1.00
[(D×J)×D] S ₆ 4102		4.4 ± 0.69	8.2 ± 1.41	16.9 ± 1.88	14.7 ± 1.53	8.7 ± 1.54	9.1 ± 1.26	10.3 ± 1.58	51.9 ± 2.24	44.5 ± 2.52	19.2 ± 0.58
[(J×BB)×BB] S ₅ 4105			2.9 ± 0.62	18.8 ± 2.10	11.3 ± 1.21	7.4 ± 0.79	6.3 ± 0.78	6.8 ± 0.76	66.5 ± 3.34	43.4 ± 2.34	19.4 ± 0.58
[(D×RC)×D] S ₅ 4103				19.5 ± 2.94	28.6 ± 3.82	20.1 ± 2.31	22.2 ± 2.90	18.9 ± 2.17	85.4 ± 8.33	87.1 ± 6.95	35.4 ± 1.42
[(D×RC)×D] S ₅ 4104					15.5 ± 2.48	17.0 ± 1.66	19.2 ± 2.35	17.6 ± 1.50	79.8 ± 4.71	88.6 ± 7.93	32.4 ± 1.21
Denmark, 4106						8.8 ± 1.08	10.3 ± 1.90	9.7 ± 1.13	73.3 ± 4.45	65.7 ± 5.85	24.7 ± 0.95
[(D×RC)×D] S ₆ 4107							9.4 ± 1.54	9.4 ± 1.47	68.0 ± 3.18	59.1 ± 4.13	23.6 ± 0.80
[(D×RC)×D] S ₆ 4108								10.7 ± 1.21	75.1 ± 5.61	62.5 ± 4.75	24.4 ± 0.92
[(J×RC) F ₉ 4109									118.3 ± 12.91	183.2 ± 13.32	81.9 ± 2.15
[(J×RC) F ₈ 4110										109.1 ± 11.34	76.7 ± 2.25
Grand means for number of ripe fruit per plant of inbred lines and of hybrids, respectively.										30.3 ± 1.78	35.9 ± 0.42

* Symbols: D, Denmark; J, Johannistfeuer; RC, Red Currant; BB, Bonny Best.

† Average yield lower than that of highest yielding parent.

TABLE 3
MEANS FOR WEIGHT (IN GRAMS) OF INDIVIDUAL FRUIT*

Variety or inbred line	Bounty 4101	[(D×J)×D] S ₆ 4102	[(J×BB)×BB] S ₈ 4105	[(D×RC)×D] S ₈ 4103	[(D×RC)×D] S ₈ 4104	Danmark 4106	[(D×RC)×D] S ₆ 4107	[(D×RC)×D] S ₆ 4108	(J×RC) F ₉ 4109	(J×RC) F ₈ 4110	Grand means for hybrids
Bounty, 4101	119±6.49	114±4.01	112±7.37	89±3.66	85±7.13	99±3.79	103±6.47	108±4.65	31±1.11	41±2.14	87±1.67
[(D×J)×D] S ₆ 4102		138±12.81	108±4.66	81±4.06	80±1.14	88±13.18	96±4.36	91±4.43	40±1.15	55±2.93	84±1.88
[(J×BB)×BB] S ₈ 4105			114±8.49	72±3.14	79±2.82	109±7.31	96±7.27	103±3.99	30±1.26	42±2.28	83±1.66
[(D×RC)×D] S ₈ 4103				51±2.87	51±2.87	71±3.92	70±3.97	68±4.82	22±1.05	26±1.76	61±1.15
[(D×RC)×D] S ₈ 4104				55±2.74	53±2.74	70±4.12	62±2.21	69±2.25	19±1.10	25±0.62	60±1.09
Danmark, 4106						94±6.39	91±5.06	89±4.05	27±1.47	33±1.22	75±1.99
[(D×RC)×D] S ₆ 4107							85±4.17	83±3.26	27±1.03	33±1.32	73±1.46
[(D×RC)×D] S ₆ 4108								80±3.45	26±0.80	33±1.28	74±1.19
(J×RC) F ₉ 4109									12±0.50	16±0.68	26±0.37
(J×RC) F ₈ 4110										17±0.58	34±0.58
Grand means for inbred lines and hybrids, respectively										77±1.91	66±0.44

TABLE 4
MEANS FOR NUMBER OF DAYS FROM SEEDING TO FIRST FRUIT RIPE*

Variety or inbred line	Bounty 4101	[(D×J)×D] S ₆ 4102	[(J×BB)×BB] S ₈ 4105	[(D×RC)×D] S ₈ 4103	[(D×RC)×D] S ₈ 4104	Danmark 4106	[(D×RC)×D] S ₆ 4107	[(D×RC)×D] S ₆ 4108	(J×RC) F ₉ 4109	(J×RC) F ₈ 4110	Grand means for hybrids
Bounty, 4101	142±0.596	137±0.702	138±0.537	134±0.433	135±0.626	136±0.737	137±0.060	137±0.746	126±0.543	128±0.879	134±0.238
[(D×J)×D] S ₆ 4102		141±1.334	135±0.912	132±0.795	132±0.539	138±1.097	135±0.803	135±0.957	124±0.800	126±0.809	133±.282
[(J×BB)×BB] S ₈ 4105			142±1.130	134±0.365	135±0.847	137±0.611	137±0.883	136±0.516	123±0.763	126±0.448	133±.227
[(D×RC)×D] S ₈ 4103				134±0.772	128±1.012	133±0.989	133±0.506	136±0.733	123±0.775	126±0.859	131±.253
[(D×RC)×D] S ₈ 4104					134±0.632	134±1.120	134±0.879	135±0.442	122±0.482	122±0.539	131±.253
Danmark, 4106						137±1.159	136±1.033	136±0.792	121±1.521	125±0.646	133±.329
[(D×RC)×D] S ₆ 4107							137±0.680	136±0.646	121±0.646	126±0.554	133±.269
[(D×RC)×D] S ₆ 4108								136±0.554	122±1.218	128±0.778	133±.267
(J×RC) F ₉ 4109									124±0.841	118±0.476	122±.290
(J×RC) F ₈ 4110										124±1.044	125±0.228
Grand means for inbred lines and hybrids, respectively										135±0.288	131±0.084

* Symbols: D, Danmark; J, Johannisfeuer; RC, Red Currant; BB, Bonny Best.

TABLE 5
MEANS FOR NUMBER OF DAYS FROM FIRST FRUIT SET TO FIRST FRUIT RIPE*

Variety or inbred line	Bounty 4101	[(D×J)×D] S ₆ 4102	[(J×BB)×BB] S ₈ 4105	[(D×RC)×D] S ₈ 4103	[(D×RC)×D] S ₈ 4104	Danmark 4106	[(D×RC)×D] S ₆ 4107	[(D×RC)×D] S ₆ 4108	(J×RC) F ₉ 4109	(J×RC) F ₈ 4110	Grand means for hybrids
Bounty, 4101	54±0.471	52±0.327	50±0.221	40±0.327	50±0.233	53±0.504	52±0.267	51±0.340	43±0.213	45±0.379	49±0.108
[(D×J)×D] S ₆ 4102		51±0.512	50±0.367	48±0.394	49±0.416	53±0.447	51±0.611	51±0.494	43±0.559	43±0.307	49±0.150
[(J×BB)×BB] S ₈ 4105			51±0.277	47±0.340	50±0.428	51±0.221	50±0.379	50±0.201	41±0.277	42±0.291	48±0.107
[(D×RC)×D] S ₈ 4103				46±0.300	46±0.200	50±0.563	49±0.314	50±0.327	41±0.327	43±0.423	47±0.123
[(D×RC)×D] S ₈ 4104					48±0.537	51±0.407	50±0.335	50±0.240	40±0.409	45±0.340	48±0.119
Danmark, 4106						53±0.433	52±0.395	52±0.180	45±0.482	45±0.260	50±0.134
[(D×RC)×D] S ₆ 4107						51±0.348	51±0.473	51±0.473	43±0.300	45±0.267	49±0.128
[(D×RC)×D] S ₆ 4108								51±0.427	43±0.422	45±0.306	49±0.122
(J×RC) F ₉ 4109									38±0.458	40±0.482	42±0.137
(J×RC) F ₈ 4110										42±0.359	44±0.121
Grand means for inbred lines and hybrids, respectively										48±0.133	48±0.040

TABLE 6
MEANS (IN CENTIMETERS) FOR SPREAD OF PLANT*

Variety or inbred line	Bounty 4101	[(D×J)×D] S ₆ 4102	[(J×BB)×BB] S ₈ 4105	[(D×RC)×D] S ₈ 4103	[(D×RC)×D] S ₈ 4104	Danmark 4106	[(D×RC)×D] S ₆ 4107	[(D×RC)×D] S ₆ 4108	(J×RC) F ₉ 4109	(J×RC) F ₈ 4110	Grand means for hybrids
Bounty, 4101	78±1.904	80±2.432	92±3.815	92±2.390	88±2.782	83±2.609	81±1.886	79±1.893	120±3.614	91±2.604	90±0.915
[(D×J)×D] S ₆ 4102		69±2.859	86±3.235	83±1.978	91±2.156	75±2.807	74±1.542	78±2.744	115±2.378	84±1.718	85±0.796
[(J×BB)×BB] S ₈ 4105			73±2.899	92±2.680	102±2.481	87±2.128	84±3.224	85±3.101	112±2.205	99±3.067	93±0.979
[(D×RC)×D] S ₈ 4103				81±3.077	93±1.103	78±3.021	81±2.712	82±2.334	121±3.259	99±2.335	91±0.832
[(D×RC)×D] S ₈ 4104					90±1.721	90±3.609	91±3.163	90±1.893	125±3.195	95±2.376	96±0.876
Danmark, 4106						76±2.785	73±2.812	75±3.273	120±3.133	91±2.720	86±0.977
[(D×RC)×D] S ₆ 4107							71±2.151	73±2.538	115±2.770	91±2.698	85±0.882
[(D×RC)×D] S ₆ 4108								73±2.663	110±2.983	92±1.305	86±0.844
(J×RC) F ₉ 4109									100±2.795	122±1.472	118±0.952
(J×RC) F ₈ 4110										78±2.539	96±0.778
Grand means for inbred lines and hybrids, respectively										79±0.815	93±0.280

* Symbols: D, Danmark; J, Johannisfeuer; RC, Red Currant; BB, Bonny Best.

TABLE 7
MEANS FOR NUMBER OF LOCULES PER FRUIT*

Variety or inbred line	Bounty 4101	[(D×J)×D] S ₆ 4102	[(J×BB)×BB] S ₅ 4105	[(D×RC)×D] S ₅ 4103	[(D×RC)×D] S ₅ 4104	Danmark 4106	[(D×RC)×D] S ₆ 4107	[(D×RC)×D] S ₆ 4108	(J×RC) F ₉ 4109	(J×RC) F ₈ 4110	Grand means for hybrids
Bounty, 4101	11.7 ± 0.499	10.4 ± 0.372	8.6 ± 0.302	9.2 ± 0.329	8.8 ± 0.280	9.7 ± 0.338	10.2 ± 0.417	8.9 ± 0.268	4.7 ± 0.121	7.3 ± 0.240	8.6 ± 0.103
[(D×J)×D] S ₆ 4102	16.1 ± 0.438	16.1 ± 0.438	9.0 ± 0.215	8.4 ± 0.204	8.0 ± 0.306	9.5 ± 0.256	9.4 ± 0.241	9.3 ± 0.370	9.6 ± 0.200	13.2 ± 0.306	9.6 ± 0.004
[(J×BB)×BB] S ₅ 4105	7.0 ± 0.150	7.0 ± 0.150	7.0 ± 0.150	7.3 ± 0.154	7.8 ± 0.161	7.8 ± 0.176	8.4 ± 0.338	7.5 ± 0.360	3.9 ± 0.090	6.4 ± 0.187	7.2 ± 0.082
[(D×RC)×D] S ₅ 4103				7.0 ± 0.270	5.9 ± 0.154	8.1 ± 0.468	8.0 ± 0.158	7.8 ± 0.243	3.7 ± 0.085	6.1 ± 0.175	7.2 ± 0.082
[(D×RC)×D] S ₅ 4104					5.8 ± 0.203	7.2 ± 0.176	6.3 ± 0.150	6.3 ± 0.132	3.8 ± 0.116	5.2 ± 0.249	6.4 ± 0.067
Danmark, 4106						8.0 ± 0.335	8.7 ± 0.357	8.0 ± 0.233	4.7 ± 0.251	6.7 ± 0.355	7.8 ± 0.104
[(D×RC)×D] S ₆ 4107						9.5 ± 0.404	9.5 ± 0.404	8.4 ± 0.299	4.5 ± 0.102	6.9 ± 0.192	7.9 ± 0.091
[(D×RC)×D] S ₆ 4108								8.4 ± 0.145	4.3 ± 0.141	6.3 ± 0.186	7.4 ± 0.087
(J×RC) F ₉ 4109									3.9 ± 0.058	6.0 ± 0.226	5.0 ± 0.055
(J×RC) F ₈ 4110										9.6 ± 0.328	7.1 ± 0.081
Grand means for number of locules per fruit in inbred lines and hybrids, respectively										8.7 ± 0.099	7.4 ± 0.027

TABLE 8
MEANS OF WEIGHT (IN GRAMS) PER LOCULE*

Variety or inbred line	Bounty 4101	[(D×J)×D] S ₆ 4102	[(J×BB)×BB] S ₅ 4105	[(D×RC)×D] S ₅ 4103	[(D×RC)×D] S ₅ 4104	Danmark 4106	[(D×RC)×D] S ₆ 4107	[(D×RC)×D] S ₆ 4108	(J×RC) F ₉ 4109	(J×RC) F ₈ 4110	Grand means for hybrids
Bounty, 4101	10.6 ± 0.751	10.6 ± 0.514	14.2 ± 1.182	10.1 ± 0.704	10.5 ± 1.084	10.4 ± 0.640	11.1 ± 1.099	12.6 ± 0.522	6.5 ± 0.239	5.9 ± 0.369	10.2 ± 0.259
[(D×J)×D] S ₆ 4102		9.2 ± 0.883	12.3 ± 0.697	9.8 ± 0.361	10.1 ± 0.350	9.5 ± 1.606	10.3 ± 0.520	10.2 ± 0.741	4.2 ± 0.162	4.2 ± 0.227	9.0 ± 0.235
[(J×BB)×BB] S ₅ 4105			17.4 ± 1.219	10.0 ± 0.411	13.2 ± 0.503	14.3 ± 0.744	11.7 ± 0.941	14.2 ± 0.947	7.8 ± 0.421	6.7 ± 0.399	11.6 ± 0.248
[(D×RC)×D] S ₅ 4103				8.0 ± 0.488	9.2 ± 0.526	9.3 ± 0.499	8.7 ± 0.482	9.2 ± 0.695	5.8 ± 0.287	4.4 ± 0.228	8.5 ± 0.164
[(D×RC)×D] S ₅ 4104					9.5 ± 0.562	10.0 ± 0.619	9.9 ± 0.349	11.0 ± 0.388	5.2 ± 0.361	4.9 ± 0.305	9.3 ± 0.183
Danmark, 4106						11.8 ± 0.929	10.4 ± 0.748	11.5 ± 0.553	5.8 ± 0.420	5.0 ± 0.311	9.6 ± 0.256
[(D×RC)×D] S ₆ 4107							9.4 ± 0.541	9.8 ± 0.592	6.0 ± 0.442	4.9 ± 0.228	9.2 ± 0.215
[(D×RC)×D] S ₆ 4108								9.5 ± 0.440	6.0 ± 0.271	5.4 ± 0.251	10.0 ± 0.197
(J×RC) F ₉ 4109									3.0 ± 0.139	2.7 ± 0.125	5.6 ± 0.099
(J×RC) F ₈ 4110										1.8 ± 0.088	4.9 ± 0.094
Grand means for inbred lines and hybrids, respectively										9.0 ± 0.218	8.8 ± 0.064

* Symbols: D, Danmark; J, Johannseuer; RC, Red Currant; BB, Bonny Best.

The data furnish some information as to whether the inbred lines with superior combining ability can be selected on the basis of the number of ripe fruit per plant of the inbred lines. On the basis of number of ripe fruit per plant, lines 4109, 4110, 4103, and 4104 would have been selected as outstanding. Table 2 shows that these inbred lines also produced the greatest number of ripe fruit per plant in hybrid combinations. This is particularly true of the intraclass hybrids.

WEIGHT OF INDIVIDUAL FRUIT

The data on weight of individual fruit are given in table 3. The figures show that the grand mean for the inbred lines is greater than the grand mean for the F_1 hybrids and also that the grand means of the hybrids of the inbred lines of classes 1 and 3 are less than the means of their respective inbred lines. The reverse is true for the same comparisons involving classes 2 and 4. Since classes 1 and 3 are composed of the larger-fruited inbred lines, these facts indicate at least partial dominance of smaller fruit.

Examination of the data in more detail reveals that the intraclass hybrids of class 1 show nonbeneficial heterosis; those of classes 3 and 4 show partial dominance of larger fruit; and the two inbred lines of class 2 do not differ sufficiently to permit conclusions. The inbred lines of class 1 do not all behave the same as regards interclass hybrids. The interclass hybrids of 4102 with the inbred lines of classes 2, 3, and 4 all show partial dominance of smaller fruit; those of 4101 and 4105 with the inbred lines of classes 2 and 3 show no dominance, or at best slight partial dominance of larger fruit. These latter inbred lines of class 1 when crossed with those of class 4 show marked partial dominance of smaller fruit. Another important fact can be

deduced from the data for the interclass hybrids derived by crossing the inbred lines of class 1 with those of classes 3 and 4; namely, the fruit of the hybrids resulting from crossing inbred lines 4101 and 4105 of class 1 with those of class 3 are larger on an average than those resulting from crossing inbred 4102 with these same inbred lines of class 3. The reverse is true for the same comparisons involving the interclass hybrids of classes 1 and 4. This interaction is well established statistically. The interclass hybrids involving the inbred lines of classes 2 and 3 show no dominance, or at most a slight partial dominance, of smaller fruit. The interclass hybrids between inbred lines of classes 2 and 4 and those between inbred lines of classes 3 and 4 show marked partial dominance of smaller fruit. From these data it can be seen that the F_1 hybrids vary from no dominance, or at most slight partial dominance, of larger fruit, to slight partial dominance of smaller fruit, to marked partial dominance of smaller fruit, to nonbeneficial heterosis. This variation is great enough to have considerable influence on yield, which is significant when it is realized that weight of individual fruit multiplied by number of fruit gives yield.

As would be expected, the hybrids with the larger fruit are derived from those inbred lines having larger fruit.

MEANS FOR NUMBER OF DAYS FROM SEEDING TO FIRST FRUIT RIPE

The means for number of days from seeding (April 17) to first fruit ripe are given in table 4. The grand mean for the inbred lines is somewhat greater than the grand mean for the F_1 hybrids, indicating heterosis for earliness of maturity, or at least complete dominance for this character in a majority of the hybrids. This is also indicated by the grand

means of the hybrids compared with the means of the inbred lines, as in every case but one the mean of the respective inbred line is greater than that of the grand mean of the hybrids. For the inbred line 4110 the reverse is true. However, the difference between the grand mean of the hybrids and the mean of the inbred line 4110 is not statistically significant. The intraclass hybrids of classes 1, 2, and 4 exhibit heterosis for earliness of maturity, whereas those of 3 do not. With the possible exception of 4102 crossed with the inbred lines of class 2, the interclass hybrids involving classes 1, 2, and 3 exhibit complete dominance for earliness. The two hybrid exceptions show heterosis. On the whole, the interclass hybrids involving 4109 tend to show a slight degree of heterosis for earliness, whereas those involving 4110 tend to show a degree of partial dominance that approaches complete dominance for earliness of maturity. These considerations show that again the character in the F_1 hybrids varies, ranging from partial dominance for earliness in some to heterosis in others. The F_1 hybrids 4101×4105 and 4109×4110 were 6 days earlier than their parents.

The difference between the earliest and the latest maturing variate is 24 days. The fact that the later inbred lines are earlier than such commonly grown varieties as Bonny Best and Earliana gives added significance to this statement. Also, in the majority of the cases earliness of maturity is completely dominant or shows heterosis. In general, the earlier hybrids resulted from crosses involving the earlier inbred lines.

MEANS FOR NUMBER OF DAYS FROM SEEDING TO FIRST BLOOM

The conclusions concerning dominance and heterosis that can be drawn from

the data for number of days from seeding to first bloom are almost identical with those for number of days from seeding to first fruit ripe. As will be shown later, this does not necessarily mean that the variation in number of days from seeding to first fruit ripe is predominantly or largely controlled by number of days from seeding to first bloom.

NUMBER OF DAYS FROM FIRST BLOOM TO FIRST FRUIT SET

Number of days from first bloom to first fruit set is not nearly so important in determining earliness and yield under the environmental conditions encountered at Cheyenne, Wyoming, as it is under the environments of some other areas. The grand means for the hybrids compared with the grand mean and means of the inbred lines show that on an average the hybrids exhibit partial dominance, complete dominance, or heterosis for the shorter period from bloom to first fruit set. Again, comparisons between the hybrids involving 4109 and those involving 4110 were interesting. In every case but one the number of days from first bloom to first fruit set was less for those hybrids having 4110 as a parent than the same period for either of the respective inbred parents. As regards the hybrids having 4109 as one of the parents, again in every case but one the values of the F_1 hybrids fell between those of the respective inbred parents or showed heterosis. In both instances the exceptions involved the hybrid 4110×4109 . The value for this hybrid was not materially different from that of the inbred parent 4109, indicating complete dominance of the longer period. These data show that differences in number of days from first bloom to first fruit set do occur among the inbred lines and that these lines differ also in their expressions of

dominance and heterosis. The range in expression is from complete dominance of the longer period from bloom to first fruit set to heterosis of the shorter period from bloom to first fruit set.

Again it was found that the inbred lines having superior combining ability could be selected on the basis of their behavior.

NUMBER OF DAYS FROM FIRST FRUIT SET TO FIRST FRUIT RIPE

The data for mean number of days from first fruit set to first fruit ripe are given in table 5. The grand mean for inbred lines and the grand mean for hybrids are equal, showing that for this stage of development—on an average—the hybrids do not have advantage over the inbred lines. The grand means for hybrids compared with the means of the respective inbred lines indicate that as regards dominance and heterosis all hybrids do not behave alike; hence, a more detailed study of the data is essential.

A comparison of the interclass hybrids of classes 1 and 2 with those of 2 and 3 furnishes some information concerning combining ability. The data for the inbreds of classes 1 and 3 and for the interclass hybrids of these inbred lines crossed with 4103 of class 2 are given below:

INBREDS OF CLASS		F ₁ HYBRIDS INVOLVING 4103 AND INBRED LINES OF CLASS	
1	3	1	3
Days	Days	Days	Days
54 ± 0.471	53 ± 0.433	49 ± 0.327	50 ± 0.563
51 ± .512	51 ± .348	48 ± .394	49 ± .314
51 ± 0.277	51 ± 0.427	47 ± 0.340	50 ± 0.327

The number of days from first fruit set to first fruit ripe was 46 ± 0.300 for the inbred line 4103. It can be seen

from the tabulated data that, even though the means for the inbreds of classes 1 and 3 are identical within reasonable limits of sampling error, the differences between the comparable means of the F₁ hybrids listed under classes 1 and 3 cannot be accounted for by the deviations expected among random samples. These facts, in terms of expression of dominance, may be stated as follows. The hybrids resulting from crossing 4103 with the inbred lines of class 1 show partial dominance for the shorter period from first fruit set to first fruit ripe, whereas the hybrids from crossing this same inbred line with the inbred lines of class 3 show partial dominance for the longer period from first fruit set to first fruit ripe. Here is a clear case of difference in combining ability which should be examined in the light of the researches of HARLAND (7, 8, 9, 10). He found a mutation "crinkled dwarf" in *Gossypium barbadense* which was recessive to the normal condition. Normal in the F₁ hybrids between some strains of *G. barbadense* and certain strains of *G. hirsutum* showed incomplete dominance over crinkled, and in the F₂ generation a whole series of types appeared, in some of which normal was nearly recessive, in others intermediate, and in still others completely dominant. Hence, the dominance relations of normal and crinkled are controlled by a system of modifying genes. Also, HARLAND found that the dominance of normal or wild type over crinkled is due to the existence of a variety of normal alleles. Some strains carry normal alleles which in a certain genetic system of modifiers are nearly completely dominant over crinkled, whereas other normal alleles—presumably in the same genetic system of modifiers—are partially recessive to crinkled. It is clear that the inferior combining ability of the

inbred lines of class 3 as compared with those of class 1 can be due to differences in genetic systems of modifiers, differences in alleles, or both. Hence, this case of difference between inbred lines in combining ability is readily explainable by the genetic information available concerning dominance and dominance modifiers (6, 20).

Similar results to those shown by the tabulated data of the preceding paragraph were obtained with comparable hybrids between inbred line 4104 and the inbred lines of classes 1 and 3. Also, from table 5 it can be seen that the interclass hybrids between the inbred lines of classes 2 and 3 show partial dominance for the longer period, whereas the interclass hybrids between the inbred lines of classes 3 and 4 show it for the shorter period. A difference in degree of partial dominance for the shorter period is shown by comparing the values of the interclass hybrids involving inbred lines of classes 3 and 4 with the values for the hybrids involving inbred lines of classes 1 and 4. Both groups of hybrids exhibit partial dominance for the shorter period, but this developmental stage is longer in the latter. The values for the intraclass hybrids of classes 1 and 2 indicate that complete dominance, or even heterosis, may occur for fewer days from first fruit set to first fruit ripe.

In summary, it can be said that the expression of dominance and heterosis ranges from partial dominance of the greater number of days from first fruit set to first fruit ripe to complete dominance—or perhaps even a slight degree of heterosis—for fewer days from first fruit set to first fruit ripe. The hybrids with fewer days from first fruit set to first fruit ripe were derived from the inbred lines having this same characteristic.

SPREAD OF PLANT

The data on spread of plant are given in table 6. The grand means show that in general the F_1 hybrids exhibited heterosis for increased spread of plant. The hybrids within class 3 did not exhibit heterosis, whereas the intraclass hybrids of all the other classes did. With the possible exception of 4103 \times 4106, the interclass hybrids between members of classes 2 and 3 exhibited complete dominance for greater spread of plant. An interesting comparison may be made between the grand mean of the hybrids involving inbred lines 4104 and the grand mean of those involving 4110 as compared with the means of these two inbred lines. The mean spread of plant of 4104 is considerably greater than that of 4110, but the two grand means for the hybrids are equal.

The expression of spread of plant in the F_1 hybrids ranges from that of partial dominance for greater spread to a rather marked degree of heterosis. In general, the inbred lines having the greatest spread of plant produce those F_1 hybrids superior in this respect.

HEIGHT OF PLANT

The conclusions drawn from the data for height of plant are so similar to those for spread of plant as not to warrant special discussion.

NUMBER OF LOCULES PER FRUIT

The data on number of locules per fruit are given in table 7. The grand mean for the inbred lines is seen to be greater than that for the hybrids, indicating partial dominance for fewer locules per fruit. That such probably is the case is also borne out by the grand means of the hybrids as compared with the means of their respective inbred lines.

This conclusion is found to hold for all intraclass hybrids, with the possible exception of 4101×4102 , and for all interclass hybrids involving classes 1, 2, and 3. The following deductions can be made regarding the interclass hybrids involving inbred line 4109. Those hybrids resulting from crossing with the inbred lines of class 1 range from complete dominance of fewer locules per fruit to no dominance; those resulting from crossing with inbred lines of class 2 show nonbeneficial heterosis; and those resulting from crossing with inbred lines of class 3 show partial dominance of fewer number of locules per fruit. In the interclass hybrids involving 4110, it can be seen that—with the exception of the hybrid 4110×4102 —all show nonbeneficial heterosis. The hybrid 4110×4102 shows no dominance. The hybrids involving 4102 also exhibit a nonuniform behavior pattern. The hybrid 4101×4102 shows slight nonbeneficial heterosis or no dominance; the hybrids resulting from crossing with inbred lines of classes 2 and 3 show partial dominance of fewer locules; and the hybrids resulting from crossing with inbred lines of class 4 show no dominance. The expression of number of locules per fruit varies from no dominance to nonbeneficial heterosis of fewer locules per fruit.

WEIGHT PER LOCULE

The data on weight per locule are given in table 8. Probably one of the most striking features of the data is the range in expression of the character. The weight per locule varies from 1.8 gm. for inbred line 4110 to 17.4 gm. for line 4105. The interclass hybrids involving the inbred lines 4109 and 4110 (with the exception of 4103×4109) showed partial dominance of smaller weight per locule. The hybrids resulting from cross-

ing inbred line 4101 with inbred lines 4107 and 4108, and those resulting from crossing inbred line 4102 with inbred lines 4107 and 4108, exhibited a slight degree of heterosis. The differences in magnitude of the means of these inbred lines were slight. Where the differences between the means of the inbred lines were marked, the resulting hybrid or hybrids generally exhibited partial dominance of less weight of locule per fruit.

The range in expression of weight per locule in the F_1 hybrids was from partial dominance of less weight per locule to heterosis of greater weight per locule. The F_1 hybrids with the greater weight per locule were derived from those inbred lines having greater weight per locule.

RELATION BETWEEN YIELD, NUMBER OF RIPE FRUIT PER PLANT, AND SIZE OF FRUIT

The future trend in methods of breeding tomatoes will be determined largely by the relation between yield, number of ripe fruit, and size of fruit. If the genetic relations and the manner of inheritance of the genes differentiating the characters are such that those giving the desired results can be easily and quickly recombined into individual inbred lines, then F_1 hybrids will have little if any importance in the commercial production of tomatoes, because it will be easier to grow the crop from inbred lines in which the desirable characters have been attained. On the other hand, if the genetic relations and the manner of inheritance of the genes differentiating the characters are such that they cannot be easily and quickly recombined into individual inbred lines, then the use of F_1 hybrids may become of increasing importance.

The relation between yield and the

two characters—number and size of fruit—is clearly defined, as yield is the product resulting from multiplying the value of one of these latter two characters by that of the other. When such a numerical system prevails, the expression of dominance and heterosis for the two independent characters gains added importance in the production of tomatoes from F_1 hybrids, because moderate increases in either number or size of fruit result in substantial increases in the dependent character yield. Likewise, moderate improvement of these two characters in the inbred lines may be expected to result in substantial increases in the yielding ability of the inbred lines themselves. An understanding of the numerical system involved emphasizes the importance of working with the component characters of yield, thereby permitting a more reliable evaluation of the gains made in either component. In turn, this makes the breeding program more flexible by allowing judicial shifts in the emphasis placed upon either of the components; hence the entire program is facilitated.

Probably one of the most important problems is the determination of the possibilities for the continued improvement of inbred lines and hybrids by increasing number of fruit and size of fruit. It is apparent that the considerable number (183) of ripe fruit per plant of the hybrid 4110×4109 cannot be recombined with the larger size (94 gm.) of fruit possessed by Denmark, owing to the physiological and morphological limitations of these plants. However, it is desirable to know whether the limit of recombination of greater number of fruit and larger size has been reached in the inbred lines and hybrids. To be more specific, can the number of ripe fruit the size of those of Denmark be materially

increased? Data bearing on this question are tabulated below:

Character	Denmark		F_1 , 4110×4109		Theoretical F_1 hybrid or inbred line
Yield (gm.).....	828.0	108.0	2876.0	177.0	2876.0
Size (gm.).....	94.0	6.39	16.0	0.68	94.0
Number.....	8.8	1.08	183.2	13.32	30.6

The actual yields, weights of individual fruit, and numbers of fruit are given for Denmark and the F_1 hybrid 4110×4109 , whereas the values for the theoretical F_1 hybrid or inbred line are based on the yield of the F_1 hybrid 4110×4109 and the fruit size of Denmark. From a consideration of this tabulation it seems that F_1 hybrids or inbred lines can be obtained which will combine the fruit size of Denmark with a considerably increased number of ripe fruit. Such F_1 hybrids or inbred lines would be superior to Denmark in yielding ability.

However, unfavorable linkage, pleiotropy, number of gene pairs differentiating the contrasted characters, or a combination of the three may make such an accomplishment exceedingly difficult or practically impossible. Information bearing on whether such is the case can be obtained by considering the advances already made. Some of the more pertinent data are tabulated below:

Character	F_1 , 4110×4102		F_1 , 4101×4103	
Yield (gm.)....	2428.0	150.0	1827.0	196.0
Size (gm.).....	55.0	2.93	89.0	3.66
Number.....	44.5	2.52	20.5	2.27

These values should be compared with those of Denmark and of the F_1 hybrid 4110×4109 (given in the preceding tabulation). The number of fruit that ripened in hybrid 4110×4102 is approximately five times that of Denmark,

and the weight of individual fruit is approximately four times that of the F_1 hybrid 4110 \times 4109. Also, the size of fruit of the F_1 hybrid 4101 \times 4103 is not significantly different from that of Danmark, whereas the number of fruit that ripened is more than twice that of Danmark. Although decided advances have been made in combining increased number and size of fruit in inbred lines, they are not nearly so impressive as those made in combining these two desirable characters in the F_1 hybrids.

Probably equally pertinent evidence bearing on the problem as to how much progress can be made in combining more and larger fruits is furnished by a consideration of the advances that have been made in breeding superior inbred lines. Inbred lines 4108 and 4110 were crossed in an attempt to increase size of fruit and increase or at least maintain earliness. From the F_2 and first generation backcrosses, a number of individuals were selected which fulfilled these requirements. Also, crosses were made between Danmark and Ponderosa in an endeavor to obtain inbred lines that matured as early as Danmark and possessed considerably larger fruits. From some of the backcross progenies which had been inbred by self-pollination for four generations after the original backcross, strains were selected in 1942 which were as early maturing as Danmark and which had fruits one and one-half times as large. F_1 hybrids involving some of these improved strains should have fruits of acceptable commercial size and in addition should produce considerably greater yields than any of the commercial varieties available at the present time.

That the numerical system clearly defines the relation between yield and the two characters number of ripe fruit and size of fruit has been shown. Since num-

ber of ripe fruit multiplied by weight of individual fruit gives yield, all the variability (variance) of yield must be accounted for by the multiple regression of yield on number of ripe fruit and size of fruit. The relative influence of these two characters on yield has not been determined; that is, what percentage of the variation attributable to yield has been accounted for by number of ripe fruit and what percentage by weight of individual fruit. Again, regression would be expected to yield some information.

Before going into an interpretation of the data, some facts should be mentioned. It will be remembered that the design of the experiment was that of a randomized complete block, composed of ten blocks with fifty-five variates (hybrids and inbred lines) per block; hence the sources of variation are as shown in table 9. The variation between means of hybrids and inbred lines is largely genetic; that due to blocks is mainly environmental; and that due to interaction may be both. Also, in the original cross from which seven of the ten inbred lines resulted, numerous fruit and small size were combined in one parent and fewer fruit and larger size in the other. Even though much progress has been made toward recombining these two desirable characters, comparatively speaking greater number of ripe fruit and smaller size still tend to be associated. Then, even though the maximum limit of recombination of greater number of fruit and larger size has not been attained in the hybrids and inbred lines, a negative relation might be expected between these two characters.

The percentages of the variances of weight of all fruit that ripened per plant (yield), number of fruit that ripened per plant, and weight of an individual fruit (size) that can be accounted for by re-

gression are given in table 9. When the association between the characters is negative, the respective values are followed by a negative sign in parentheses. All values were transformed to loga-

TABLE 9
PERCENTAGE OF VARIANCES OF WEIGHT OF RIPE
FRUIT PER PLANT (YIELD), OF NUMBER OF
FRUIT THAT RIPENED PER PLANT (NUMBER),
AND OF WEIGHT OF AN INDIVIDUAL FRUIT
(SIZE) THAT ARE ACCOUNTED FOR BY REGRES-
SION

INDEPENDENT CHARACTERS	DEPENDENT CHARACTERS		
	Simple regression		Multiple regression
	Size (%)	Yield (%)	Yield (%)
Main effects			
Hybrids and in- breds			
Number.....	89.82 (-)	84.95	99.98
Size.....		56.20 (-)	
Blocks			
Number.....	0.38	95.82	99.99
Size.....		6.97	
Interaction			
Hybrids and in- breds X blocks			
Number.....	0.26 (-)	71.09	99.97
Size.....		24.35	
Total			
Number.....	67.93 (-)	71.21	99.99
Size.....		15.35 (-)	

arithms for calculating regressions. Considering the variation attributable to differences between hybrids and inbreds, the table shows that 89.82% of the variance of number of fruit and size of fruit are accounted for by the regression of these two characters upon each other. The relation is negative. Eighty-four and

ninety-five hundredths per cent of the variance of yield is accounted for by the regression of yield on number of fruit and 56.20% by yield on size. In the first case the association is positive and in the latter negative. The multiple regression of yield on the other two characters accounted for practically all (99.98%) the variability of yield. This shows that for all practical purposes the correct transformation of the data was used. The fact that the association between size of fruit and yield is negative, whereas the association between number of ripe fruit and yield is positive, indicates that in this material number of ripe fruit has a greater influence in bringing about an increase in yield than size of fruit. The negative association between number of ripe fruit and size of fruit seems to be due to the way these two characters were combined in the original parents and inbred lines, rather than to any other genetic or physiological phenomena. From the data listed under blocks it is evident that all the relations are positive and that number of ripe fruit per plant accounts for a considerably greater proportion of the variance of yield than does size of fruit. Also, the number of ripe fruit per plant and the size of fruit are practically independent of each other. Regression accounted for very little of the variance of either character. From these data, and from those given under interaction and total of table 9, the following conclusions seem logical. Indications are that number of ripe fruit per plant accounted for approximately 85% of the genetic variability due to differences in yield and about 95% of the environmental variability due to differences in this character; whereas size of fruit accounted for the remaining 15% and 5%, respectively.

The following summary concerning

the interrelation of yield per plant, number of ripe fruit, and size of fruit seems justified: It should be possible materially to increase yields of commercially acceptable tomatoes by recombining greater number of fruit that ripen with larger size of fruit. In fact, inbred lines are now available at the Cheyenne Horticultural Field Station which should make this possible if F_1 hybrids are grown for production of the commercial crop.

RELATION BETWEEN NUMBER OF RIPE
FRUIT, DAYS FROM SEEDING TO FIRST
FRUIT RIPE, SPREAD OF PLANT, AND
HEIGHT OF PLANT

The method of regression was employed also to study the relation between number of ripe fruit per plant, number of days from seeding to first fruit ripe, spread of plant, and height of plant. The variation analyzed was that due to differences between hybrids and inbred lines. Fifty-six per cent of the variance of number of ripe fruit per plant was accounted for by the regression of this character on spread of plant; 63% of the variance of number of days from seeding to fruit ripe was accounted for by the regression of this character on spread of plant; and 93% of the variance of number of ripe fruit per plant was accounted for by the regression of this character on number of days from seeding to fruit ripe. As would be expected in the latter two instances, the association was negative. Apparently, spread of plant influences number of ripe fruit. Also, earliness of maturity would be expected to have a decided influence on number of fruit that ripen in locations such as Cheyenne, Wyoming, where freezes and frosts occur early. The results for height of plant were very similar to those for spread of plant and hence need not be considered further. The percentage of the variance of number of ripe fruit

accounted for by multiple regression is very little greater than that accounted for by the regression of this character on earliness of maturity. Then it would seem that size of plant as determined by measurement of spread of plant and height of plant may be having an effect on number of ripe fruit per plant by increasing earliness of maturity.

If these conclusions are correct, one might expect to find some hybrids and inbred lines that combine greater number of ripe fruit per plant, earliness of maturity, and greater plant spread. That these three desirable characters do occur in some F_1 hybrids and inbred lines is shown by the data in columns 4109 and 4110 of tables 2, 4, and 6. The possibilities for increasing yield by combining these three characters are interesting. The fact that inbred line 4110 produced 1868 gm. of ripe fruit with a plant spread of only 78 cm. indicates that it should be possible to attain considerably higher yields by combining the plant spread (100 cm.) of 4109 with the desirable characters of 4110. The F_1 hybrid 4110 \times 4109 as compared with the inbred parents represents an increase in number of ripe fruit per plant, earliness of maturity, and plant spread which is accompanied by a decided increase in yield.

The relation between height of plant and the other characters is very similar to that for spread of plant and the other characters and therefore does not need discussion.

The relation between size of fruit and number of days from date seeded to first fruit ripe should now be considered. In 1941, studies were conducted to determine what progress had been made in recombining extreme earliness and large size of fruit. The data are summarized in table 10. They show that size of fruit as determined by weight can be increased

five to thirteen times that of the earlier parent, Red Currant, without loss of earliness. In fact, selection 13-7, which is in the F_7 generation, is somewhat earlier than Red Currant and has fruits thirteen times as large. From tables 3 and 4 it can be seen that some crosses with this same inbred line 13-7 (4109) have fruits two to three times as large as inbred line 13-7 (4109) and are just as early—or earlier—maturing. These data indicate that large size of fruit and ex-

are additive. Hence, if these characters can be recombined in various ways in inbred lines, and through these inbred lines in F_1 hybrids, then it can be concluded that the end effects of some of the genes differentiating earliness of maturity (days from seeding to first fruit ripe) are additive. The following facts can be derived from tables 4 and 5 and the following data. Inbred lines 4109 and 4110 were derived from the same cross—Johannisfeuer \times Red Currant. The means for number of days from date seeded to first fruit ripe are the same, 124 days, for both inbred lines. Likewise, the number of days from date seeded to first bloom are identical. But, comparatively speaking, the period from first bloom to first fruit set is long (9.2 ± 0.586 days) for inbred line 4109 and short (6.4 ± 0.514 days) for 4110. The period from first fruit set to first fruit ripe is shorter in 4109 than in 4110 (table 5). This one set of comparisons alone shows that these three characters can be recombined in different combinations. Also, the same conclusion can be drawn from a comparison of inbred lines 4103 and 4106. All three of the short periods of the three independent characters are combined in the F_1 hybrid, 4104 \times 4109.

Again the method of regression should add to our understanding of the relation between the characters. The data were transformed to logarithms before the calculations were made. The percentages of the variances that are accounted for by regression are given in table 11. Only the analysis of the variation due to differences between hybrids and inbreds is given. The data in table 11 support the following conclusions: The majority of the variation of the independent characters is independent; that is, not attributable to covariation. This is in accord with the previous data showing that

TABLE 10
MEAN NUMBER OF DAYS FROM SEEDING TO FIRST
FRUIT RIPE (MATURITY) AND MEAN WEIGHT
(SIZE) OF INDIVIDUAL FRUIT

Strain or hybrid*	Maturity (days)	Size (gm.)
Red Currant.....	128.3 ± 1.183	1.18 ± 0.057
J \times RC, F_1	126.0 ± 0.954	7.1 ± 0.233
J \times RC, F_7 , 13-7...	124.5 ± 0.582	16.0 ± 0.447
J \times RC, F_7 , 196-6..	126.3 ± 0.932	15.0 ± 0.471
J \times RC, F_7 , 379-5..	128.7 ± 0.895	14.2 ± 0.533
Johannisfeuer.....	141.9 ± 1.130	51.1 ± 1.841
D \times J, F_1	140.2 ± 1.209	58.2 ± 1.788
D \times RC, F_1	124.5 ± 0.991	8.6 ± 0.163
Danmark.....	148.1 ± 0.883	67.6 ± 1.851

* Symbols: RC, Red Currant; J, Johannisfeuer; D, Danmark.

treme earliness of maturity can be recombined, either in F_1 hybrids or inbred lines.

*RELATION BETWEEN DAYS FROM SEEDING
TO FIRST FRUIT RIPE, DAYS FROM SEED-
ING TO FIRST BLOOM, DAYS FROM FIRST
BLOOM TO FIRST FRUIT SET, AND DAYS
FROM FIRST FRUIT SET TO FIRST FRUIT
RIPE

Days from seeding to first fruit ripe logically may be partitioned into days from seeding to first bloom, days from first bloom to first fruit set, and days from first fruit set to first fruit ripe (16). Obviously, the numerical system is such that the values of the different characters

the desirable characters can be recombined in inbred lines and F_1 hybrid populations. Also, it seems that number of days from first fruit set to first fruit ripe

TABLE 11

PERCENTAGE OF VARIANCE OF NUMBER OF DAYS FROM SEEDING TO FIRST FRUIT RIPE (MATURITY), NUMBER OF DAYS FROM SEEDING TO FIRST BLOOM (BLOOM), NUMBER OF DAYS FROM FIRST BLOOM TO FIRST FRUIT SET (SET), AND NUMBER OF DAYS FROM FIRST FRUIT SET TO FIRST FRUIT RIPE (RIPE) THAT ARE ACCOUNTED FOR BY REGRESSION. ONLY THE ANALYSIS OF VARIATION DUE TO DIFFERENCES BETWEEN HYBRIDS AND INBREDS IS GIVEN

INDEPENDENT CHARACTERS	DEPENDENT CHARACTERS		
	Set (%)	Ripe (%)	Maturity (%)
Bloom.....	18.44 (-)	24.43	56.24
Set.....		0.56	0.14
Ripe.....			85.19
Multiple regression...			99.80

has a greater influence on number of days from seeding to first fruit ripe than do number of days from seeding to first bloom or number of days from first bloom to first fruit set. The fact that 99.8% of the variance was accounted for by the multiple regression of number of days from seeding to first fruit ripe on the independent characters shows that the transformation to logarithms placed the data on the proper scale of measurement.

RELATION BETWEEN WEIGHT OF INDIVIDUAL FRUIT, NUMBER OF LOCULES PER FRUIT, AND WEIGHT PER LOCULE

Weight of individual fruit may be partitioned into number of locules per fruit and weight per locule. Again, the numerical system is such that the effects of the two independent characters are multiplicative. Also, all the variation of

weight of individual fruit must be accounted for by variation in number of locules per fruit and weight per locule. That greater number of locules and greater weight per locule can be combined is shown by inbred lines 4101 and 4102. Line 4101 has 11.7 ± 0.499 locules per fruit which weigh 10.6 ± 0.751 gm. per locule, and line 4102 has 16.1 ± 0.438 locules per fruit which weigh 9.2 ± 0.883 gm. per locule.

The method of regression was employed to obtain further information concerning the relation between the characters. Only 10% of the variation of number of locules and weight of locules was accounted for by covariation (table 12). Therefore, of the magnitudes and range of the characters accounted for in this material there would not seem to be any appreciable physiological or morphological limitations on the recombination of these two characters. In this material the values of covariation indicate that

TABLE 12

PERCENTAGE OF VARIANCES OF WEIGHT OF INDIVIDUAL FRUIT (FRUIT SIZE), NUMBER OF LOCULES PER FRUIT (NUMBER), AND WEIGHT PER LOCULE (LOCULE SIZE) THAT ARE ACCOUNTED FOR BY REGRESSION. ONLY THE ANALYSIS OF VARIATION DUE TO DIFFERENCES BETWEEN HYBRIDS AND INBREDS IS GIVEN

INDEPENDENT CHARACTERS	DEPENDENT CHARACTERS	
	Locule size (%)	Fruit size (%)
Number.....	10.09	52.71
Locule size.....		77.64
Multiple regression.....		99.76

weight per locule is probably somewhat more influential in regulating size of fruit than is number of locules. The multiple regression of size of fruit on number of locules and weight per locule accounted for 99.76% of the variation of

size of fruit, showing that the regression is primarily logarithmic rather than linear.

Discussion

An important contribution of these studies is the information they furnish concerning the nature of combining ability. The partitioning, in so far as practical, of the main characters into their components has been particularly helpful in showing the relation between combining ability and genetic diversity, dominance, and heterosis. An extensive review of the literature concerning dominance and heterosis is not within the province of this paper. For such a review, and for some of the more pertinent facts, reference is made to GOLDSCHMIDT (6), WADDINGTON (20), WRIGHT (22), BEADLE and COONRADT (4), and POWERS (14, 15). Particularly pertinent to an understanding of the nature of combining ability is the work of HARLAND (7, 8, 9, 10) discussed earlier in this paper (p. 257). From this genetic information concerning dominance and heterosis, and since from the standpoint of physiological genetics dominance and heterosis have been shown (14, 15) to be different degrees of expression of the same phenomenon, it is clear that combining ability of inbred lines is dependent on the nature and diversity of the alleles differentiating the contrasted characters, the intraallelic and interallelic interactions of the genes, the system of modifying genes, and finally the interaction between the genotypic milieu and the environment. In brief, the combining ability of these inbred lines is dependent upon genetic diversity and the phenomenon of dominance and heterosis, and the available information concerning these genetic phenomena is sufficient to account for all the known facts concerning combining ability.

BARRONS (2) points out that commercial seed companies hesitate to enter the field of F_1 hybrid tomato-seed production owing to the difficulties and costs involved. Hence, any means of reducing this cost and these difficulties would materially facilitate the use of F_1 hybrids in commercial production. TERNOVSKI and MISSURA (18) have shown that male sterility can be induced in a certain percentage of the plants resulting from X-ray irradiation of seed. By X-raying seed of those plants found to be desirable for the production of F_1 hybrids and growing large populations, it seems that male sterile inbred lines could be established. These could then be used in producing the hybrid seed—either by insect or by hand pollination. In case insect pollination of the induced male steriles is found to be feasible, the cost and difficulties of producing hybrid seed would be greatly reduced. Even though insect pollination did not prove satisfactory, hand pollination would be much easier owing to the elimination of the necessity for emasculation. The induction of male sterility in inbred lines to be used in making F_1 hybrids is of sufficient promise to warrant intensive studies on the production of male steriles by irradiation, on determining the value of such inbreds in a tomato-breeding program, and on working out methods of utilizing them to obtain F_1 hybrid tomatoes for commercial production.

Summary and conclusions

1. The yield of ripe tomatoes in those areas having a short growing season can be greatly increased by utilizing F_1 hybrids for producing the crop. All the high producing F_1 hybrids have at least one parent derived from crosses between *L. esculentum* Mill. and *L. pimpinellifolium* (Jusl.) Mill. The most outstand-

ing F_1 hybrid yielded by weight three times as much ripe fruit per plant as did Denmark. This increased yield was due primarily to an increase in earliness. The range in expression of dominance and heterosis varied from no dominance to decided beneficial heterosis.

2. In respect to number of ripe fruit per plant, the range in expression of dominance and heterosis varied from partial dominance of fewer fruits per plant to heterosis for greater number of fruits per plant. This is significant when considering that the range in number of ripe fruit per plant varied from 2.9 to 183.

3. As regards weight of ripe fruit, the F_1 hybrids varied from no dominance—or at most, slight partial dominance—of larger fruit to slight partial dominance of smaller fruit to marked partial dominance of smaller fruit to nonbeneficial heterosis. This variation is sufficient to play considerable part in determining yield.

4. The difference between the earliest and the latest maturing variate is 24 days. That the latest maturing inbred lines and F_1 hybrids are earlier than such commonly grown varieties as Bonny Best and Earliana gives added significance to this fact. Also, in the majority of cases earliness of maturity is completely dominant or shows heterosis.

5. The range in expression of dominance and heterosis is from complete dominance of the longer period from bloom to first fruit set to heterosis of the shorter period from bloom to first fruit set.

6. For number of days from first fruit set to first fruit ripe the expression of dominance and heterosis ranges from partial dominance of the greater number of days from first fruit set to first fruit ripe to complete dominance or perhaps

even a slight degree of heterosis for fewer days from first fruit set to first fruit ripe.

7. For spread of plant the expression of the character in the F_1 hybrids ranges from partial dominance for greater spread of plant to a rather marked degree of heterosis. The conclusions for height of plant are very similar to those for spread of plant.

8. The expression of number of locules per fruit in the F_1 hybrids varies from no dominance to nonbeneficial heterosis of fewer number of locules per fruit.

9. In the F_1 hybrids the expression of weight per locule varies from partial dominance of smaller weight per locule to heterosis of greater weight per locule.

10. The interrelation of yield per plant, number of ripe fruit, and size of fruit is such that it should be possible materially to increase yields of commercially acceptable tomatoes by recombining greater number of fruit that ripen with larger size. In fact, inbred lines are now available at the Cheyenne Horticultural Field Station which should make this possible if F_1 hybrids are grown for production of the commercial crop.

11. The relation between number of ripe fruit, days from seeding to first fruit ripe, and spread of plant is such that it should be possible to obtain inbred lines and F_1 hybrids having the desired combination. Extreme earliness and large size of fruit probably can be recombined.

12. Days from seeding to first fruit ripe logically may be partitioned into days from seeding to first bloom, days from first bloom to first fruit set, and days from first fruit set to first fruit ripe. The relation between these characters is such that the shorter periods of development can be combined to produce a decidedly earlier maturing tomato.

13. The relation between number of locules per fruit and weight per locule is such that the greater number of locules and the greater weight per locule can be recombined to give increase in size of fruit.

14. In general, the data show that the inbred lines having superior combining ability—as determined by the behavior of F_1 hybrids in respect to the eleven characters studied—are themselves superior in comparison with other inbred lines. However, the exceptions to this statement are of sufficient importance so that they cannot be ignored in a plant-breeding program.

15. From these studies dealing with the degree of expression of the phenomenon of dominance and heterosis for the eleven quantitative characters, and from the information obtained by partitioning some of the main characters into their components, it is apparent that the greatest strides in breeding can be made by utilizing F_1 hybrids rather than inbred lines for the production of commercial crops.

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LEAF AND BUD INITIATION IN THE GRAMINEAE

B. C. SHARMAN

Introduction

In spite of the widespread distribution and the economic importance of the order, there appears to have been very little investigation of the mode of initiation of the leaf and bud in the Gramineae. What little literature there is fails to give an exact picture of the earliest stages: it is hoped that the present study may elucidate some of the more salient features.

DOULIOT (12), studying mainly *Phragmites communis* Trin., stated that the new primordium appears as an annular swelling encircling the stem tip. He noticed that the epidermis and the underlying cells both play a part in the production of the first up-pushing but considered that after this stage the whole leaf is derived by division of the cells forming the edge of the ring. He considered that the ring is the future sheath and that the lamina grows at the expense of the cells of the free edge or border of the sheath, growing by a terminal cell.

BARANETZKY (5), in *Bambusa arundinacea* Willd., also noticed periclinal divisions in the epidermis at the initiation of the primordium, but he was not certain that it was a constant feature.

BUGNON (11), studying *Dactylis glomerata* L. and *Melica altissima* L., showed that the new primordium originates on one side of the apex and that the periclinal divisions in the epidermis and underlying layer spread sideways from the point of origin to give the small ringlike protuberance. He stated that the ring might be regarded as the lamina and not the sheath and that it possesses a great number of terminal initials all along its free edge. He was of the opinion

that the cells underlying the epidermis themselves divide periclinally and so help to raise the original up-pushing and contribute to the tissue of the developing leaf.

ARTSCHWAGER (3) gave a drawing of a longitudinal section of the shoot apex of *Saccharum officinalis* L., but he was mainly concerned with the later development of the leaves and axis and made no mention of the mode of origin of the primordia.

RÖSLER (18) stated that in *Triticum vulgare* Host., and also in *Avena sativa* L. and *Secale cereale* L., the original protuberance (the very young primordium—*Blatthöcker*) arises solely from the dermatogen, although he did not seem completely convinced that the cells of the layer immediately inside do not contribute something during the later stages.

PORTERFIELD (17) examined the anatomy of the apex of *Phyllostachys nigra* Munro from the point of view of HANSTEIN's histogens. He does not seem to have been very certain of the way the leaves are initiated, but he was clearer than most of his predecessors about the shoot as a whole, and he also noticed the peculiar orientation of the axial cells foreshadowing the bud.

KLIEM (15) concluded that in *Avena sativa* some of the inner tissue of the leaf base is probably derived from the "corpus."

ABBE, RANDOLPH, and EINSET (1) studied the shoot apex of *Zea mays* L. in relation to the size of the leaves during a small part of the life history of the plant. They were not, however, directly concerned with the architecture of the apex or primordia.

The writer (22) regarded the leaf of *Zea mays* as derived from both the dermatogen and the cells immediately inside this layer. It was also pointed out that in the young primordium there is no distinction between sheath and lamina, and that the difference arises later, possibly owing to the different conditions under which each part is developing. Since then, in an attempt to establish the true course of events in the early stages, a study has been made of the origin of the primordia in a wide range of cereals and herbage grasses. In all the cases examined the main features were found to be the same, so that the following account which is presented for *Agropyron repens* is regarded as being applicable to any other member of the order.

Material and methods

Couch grass, *Agropyron repens* Beauv., was chosen because it has a comparatively long apex bearing a number of leaves in various stages of early development. At the same time, *Agropyron* offered the possibility of comparing the apex of an aerial shoot with that of a subterranean rhizome. Its close relationship to *Triticum* also influenced the choice.

Numerous vacuome and cytoplasmic fixatives were tried, but modifications of Chamberlain's formalin-fatty-acid-alcohol mixture gave the best results. Although it was never possible to obtain material completely without retraction of the outer wall of the dermatogen cells, the following mixture was about the best:

Propionic acid.....	6.5 cc.
Formalin (40% aqueous solution)...	10
Ethyl alcohol (70%).....	up to 100

One difficulty in the fixation of grass apices is caused by differences in the maturity of the different shoots. Although a mixture may be good for the apex of a comparatively mature shoot, it will often

cause considerable shrinking of the dermatogen cells of the apex of a newly emerged shoot. Before being fixed, the shoots had the outer leaves removed, down to and including the one with its lamina just fully exposed as far as the ligule. This ensured that the material was free from dense, heavily lignified tissue and would cut easily. It also proved useful when examining the sections, since it allowed a quick assessment of the approximate maturity of the outer few leaves.

Dehydration was carried out in absolute alcohol kept over anhydrous copper sulphate, in the manner suggested by BALL (4). Infiltration was accomplished via chloroform, since this seemed to render the tissues less hard and brittle than xylol. The absolute alcohol-chloroform mixtures were also kept over anhydrous copper sulphate.

Sections were usually cut at 4-6 μ , with the object of cutting most cells at least twice. In order to stain the cell walls of the meristematic tissues, the sections were stained—as described in more detail elsewhere (23)—in dilute aqueous safranin (1:25,000) after mordanting in a 2% aqueous solution of zinc chloride, followed by a bath consisting of 5 gm. tannic acid, 2 gm. orange G, 4 drops concentrated hydrochloric acid, and water to 100 cc. The sections were then carried through 5% tannic acid solution, followed by a 1% solution of iron-alum. As a result the cell walls were blue-black and the nuclei shades of yellow to orange, varying according to their metabolism. In actively dividing cells the cytoplasm was usually yellow or dark orange and in more quiescent cells pale yellow to grey. Procambial and vascular tissues showed good differentiation, while starch could easily be recognized, even in meristematic cells.

From time to time dissected apices were cleared in Eau de Javelle. It was found that their clarity could be greatly improved if they were taken carefully up to 50% alcohol, when they were useful for obtaining a general view of the form changes in the solid.

All drawings were made with the aid of a microprojector: in the case of longitudinal sections especially, it was usually necessary to combine two or more sections to keep the view in the appropriate plane.

Observations

MORPHOLOGY OF SHOOT

The underground rhizome produces only scale leaves, which are usually undivided into laminae and sheaths. When the soil surface is reached in spring, a succession of normal leaves is produced, beginning with a transitional leaf (usually only one) which bears a small lamina about 0.5 mm. long. Each successive leaf lamina is longer, until about the third from the inflorescence is reached—when the sizes decrease a little. Considering the transitional as the first, the main axis usually produces twelve leaves in all. There may perhaps be some slight variation in number from year to year, but during any one season about 80% of the main shoots conform to one number and the remaining 20% deviate by only one leaf more or one less.

The shoot apex of the rhizome growing horizontally underground is an elongated dome-shape, seen in figure 1A and in longitudinal section in figure 2. From this stage until the shoot bears two to three fully developed green leaves, the apex appears as in figure 1B-C', when about three to five primordia may be observed in various stages of maturity. Each primordium appears as a small protuberance on one side of the apex a little dis-

tance back from the extreme tip (fig. 1C). This is quickly transformed into a crescent and then into a collar almost completely surrounding the apex. At first the growth appears to be mainly at right angles to the surface of the axis, but it soon takes on a more vertical trend. The first-formed part is always farthest ahead, and soon the primordium forms a cowl or hood growing over and inclosing the apex (fig. 1A-D). Growth from this stage on is extremely rapid; only about two plastochrones separate it from the time when the tip of the leaf is first beginning to appear from the center of the shoot, and only three or four until the lamina is fully exposed and elongated.

As the shoot becomes more mature the apex becomes more elongated and bears more primordia (fig. 1), until—when approximately the fifth or sixth green leaf is fully exposed—there are about eight between the youngest and the one just overtopping the apex (fig. 1D, D').¹ Figure 3 shows a similar apex in longitudinal radial section, cut in the plane of the leaf insertions. Although at this stage the primordia look exactly like those which arose earlier, presumably only those at the base of the apex will develop into normal green leaves, since the total on a fully developed shoot is twelve. The rest will presumably cease development early, and at the most appear as small ridges subtending the spikelets in the inflorescence. The stage seen in figure 1D and D' is followed by

¹ It is not easy to find a natural scale for comparing one apex with another. Counting the number of successive primordia from the youngest to that just sufficiently large to inclose the younger ones and overtop the stem tip gives a simple measure for comparisons. Although this method has the obvious defect that a longer apex will need a rather more mature leaf to inclose it, this is not serious because the leaves are elongating rapidly by the time they are forming the "hood" and leaving the immediate region of the apex.

rapid elongation of the apex and inflorescence initiation, when buds develop precociously up on the apex itself and the early growth of the primordia becomes slower, so that the buds (in reality axillary buds) become the conspicuous feature and the subtending primordia in whose axils they arise no longer attract attention (fig. 1*E, F, F'*).

Underground buds grow out into new rhizomes. Under favorable conditions, the next one (or sometimes two) in the axil of the lowest aerial leaf produces a flowering shoot in the same way as the main axis. Of the remaining buds, only the lowest two or three grow out and then produce only "blind" shoots. Their axes elongate and the shoots are elevated

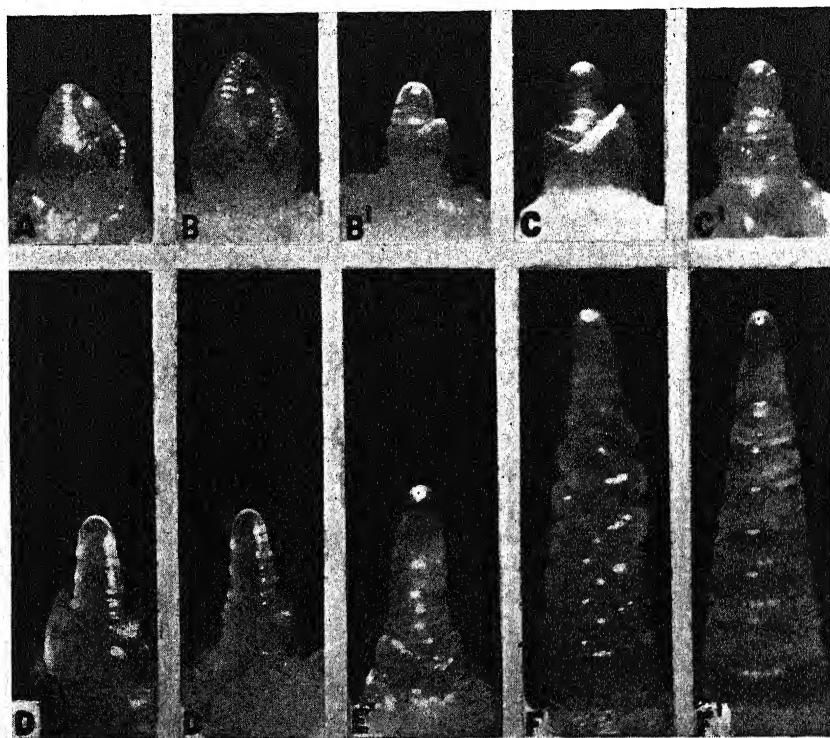


FIG. 1.—Dissected apices, mid-April to end of May (*A-C'*, vegetative; *D-F'*, just prior to and after inflorescence initiation). *A*, apex from rhizome with outer scale leaf and next four in the bud removed. *B*, from shoot with fourth leaf lamina fully exposed. *B'*, same with next outer cowl-like leaf (eighth on shoot) removed. *C*, similar apex from slightly more mature shoot. *C'*, same apex with next leaf (ninth on shoot) removed. *D*, apex just prior to inflorescence production from shoot with seventh leaf lamina fully exposed. *D'*, same with next leaf (twelfth) removed. *E*, apex at inflorescence initiation from shoot with seventh (? eighth) fully exposed leaf and four others in bud removed. *F, F'*, two views of young inflorescence with seventh (? eighth) fully exposed leaves and four others removed. See text for method of numbering.

Buds normally occur in the axils of the scale leaves and the first five green leaves. In dissected material they are not seen externally until the primordium has grown well over the top of the apex.

rather like the flowering shoots, but only a succession of leaves is developed. The apex of such shoots is small, possibly smaller than that of a rhizome, and the rate of leaf initiation seems slow. In dis-

sected material the young primordia always seem pliant and soft, as though suffering from lack of water.

DEVELOPMENTAL ANATOMY

APEX.—Clothing the tip of the apex is a single layer of anticlinally dividing cells—the dermatogen (figs. 2; 3; 5; 7*A*). Although—in view of what will be shown to be its role in leaf initiation—this layer cannot of course be regarded as a “dermatogen” in HANSTEIN’s original sense, it seems desirable to keep his term, without, however, letting it carry his implications.

Disregarding for the moment the ultimate origin of the tissues, a little way

mainly anticlinally and so giving rise to a number of strata. The layer immediately inside the dermatogen will be referred to as the hypodermis (*h*, figs. 2–6*A*; 7*A*; 9).

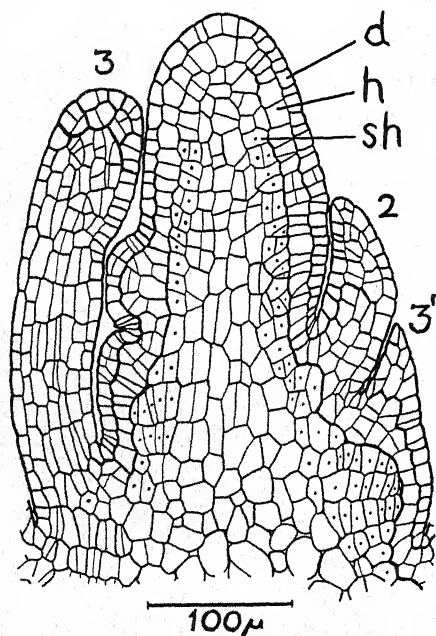


FIG. 2.—Radial longisection of apex of rhizome in plane of leaves: 2 and 3, tips of second and third primordia; 3', encircling base of third primordium, counting from tip. *d*, *h*, *sh*, dermatogen, hypodermis, and subhypodermis.

back from the apex they are found to be arranged in a definite and characteristic pattern. The outer cells are dividing

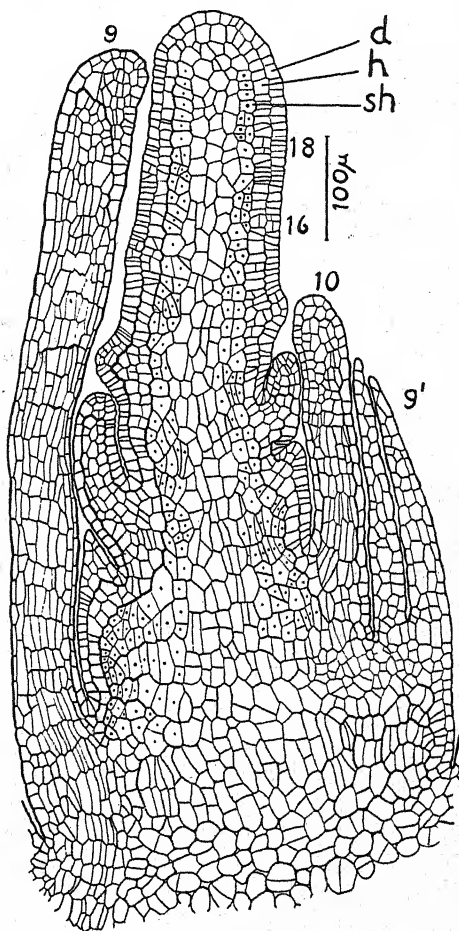


FIG. 3.—Radial longisection (in plane of leaves) of apex of shoot with laminae of about five leaves exposed. As mature shoots normally bear only twelve green leaves in all, only the basal four primordia would develop to completion and the higher ones would cease growth early. 9', two encircling overlapping wings of base of ninth leaf. *d*, *h*, *sh*, dermatogen, hypodermis, and subhypodermis.

Beneath this is a layer designated the subhypodermal layer (*sh*, figs. 2–6*A*; 7*A*; 9). In all the figures most of the cells of this layer are indicated by dots, intended to show the outer limits of this layer and

distinguish between it and the hypodermis. These dots are not intended to define the inner limits of the subhypodermis: where the distinction is clear, as in most cases, all the layer is dotted, but where the exact inner boundary is indefinite (as, for instance, toward the base of figures 2 and 3 or in figure 4K) only the outer cells are marked. In some figures it will be seen that quite a few more cells can be assigned to this layer, but it was thought better to limit the dots to the very obvious.

Inside the subhypodermal layer is a central core of rather larger, less rapidly dividing cells with less densely staining contents, forming what might be described as an "apical pith." When much starch is present, as in the rhizome, these central cells have an abundance, while only a few grains appear in occasional cells of the outer three layers. In the young and the old aerial shoots—and the rhizome as well—the part of the apex inside the dermatogen "shell" seems to originate from two initials (or groups of initials), one lying vertically above the other. This situation represents that described by RÖSLER (18) and KLIEM (15) for the older apices of *Triticum* and *Avena*, respectively. In these two species, at inflorescence production the regions called in the present study subhypodermis and core are derived from an initial or group of initials, which for long periods is distinct from that above, which is solely giving rise to the hypodermis. Both RÖSLER and KLIEM make a great point of showing that this condition in *Triticum* and *Avena* is only temporary because every now and again the hypodermal initial divides periclinally, resulting in the displacement backwards of the old initial of the "subhypodermis-plus-core" and its subsequent loss into the differentiating tissues leaving the

apical region. In *Agropyron*, however, in both the rhizome and the aerial shoot, careful examination of the median sections of series of longitudinal radial sections cut in the plane of the leaves failed to reveal periclinal divisions in the initial cell or cells of the hypodermis at the apex. The hypodermis appeared as a single unbroken layer over the apex, unless the section was off the median—when it was possible to obtain a spurious effect of periclinally divided hypodermal cells where the files running back from the apex were cut at an angle. Thus it would seem that in *Agropyron* periclinal divisions of the cells at the tip are extremely rare, so that the hypodermis approaches a morphological layer.

This difference between *Agropyron*, and *Triticum* and *Avena* may be related to the dimensions of the apex: the vegetative apex of *Agropyron* is a more massive structure and therefore may have a type of growth more related to that seen only in later stages in *Triticum* and *Avena*, when their apices have increased in bulk. It is conceivable that the interplay of factors like cell size and apical dimensions may have considerable bearing on the particular scheme followed by a given species at a particular period in its ontogeny. For this reason the question of the origin of the particular layers is not pursued further.

To summarize: the apex consists of an inner core of rather slowly dividing cells inclosed in three thimble-shaped "shells"—the subhypodermis, the hypodermis, and the dermatogen, the cells of each shell (especially those of the dermatogen) dividing mainly anticlinally. The two outer shells, the dermatogen and the hypodermis, seem to originate from separate initials or initial groups, while the subhypodermis and core are derived from a common initial or initial group.

ORIGIN OF PRIMORDIA.—In longitudinal radial sections taken in the plane of the leaves, the initiation of a new primordium is usually indicated by the appearance of periclinal divisions in one or two cells of the hypodermal layer (figs. 3; 4A; 6A) which are immediately followed by periclinal divisions in the dermatogen (figs. 2; 3; 4B; 6A, B). This stage is seen in transverse section in figures 5A, B; and 7A. It is difficult to be absolutely certain whether the cells of the hypodermis divide periclinally before those of the dermatogen, or whether the two are simultaneous. In longitudinal sections it is often possible to find divisions in the hypodermis foreshadowing the future primordium without being able to find any in the dermatogen, either in the median sections or in those on each side of them. In transverse sections, examination of a number of series did not reveal a single case where the hypodermal cells had divided without a single dermatogen cell doing likewise. Figures 5A and 7A, with a divided dermatogen cell on the flank of the future primordium, are typical of what was observed. Sections each side of that illustrated showed that this was the only dermatogen cell which had divided periclinally at this level. The fact that so many of the hypodermal cells have divided periclinally suggests that at some time earlier some of them would have divided but all the dermatogen cells would still be dividing only anticlinally. In cases like that illustrated, the sections higher up the apex (where the next higher primordium is to appear) do not throw any light on the matter, because so near the tip as this the core, subhypodermis, and hypodermis are not easily distinguished.

In *Agropyron* the primordium is usually initiated by periclinal divisions in two dermatogen cells only, when seen in

longitudinal section (from about two rows of dermatogen cells placed horizontally over each other). RÖSLER (18) suggested a depth of three cells for *Triticum*, but KLIEM (15) stated that in *Avena*, although the primordium may arise occasionally from a depth of three dermatogen cells, it usually comes from only two, and may even be derived from a single row. He elaborates the point by illustrating the slight differences in cell patterns which follow the three alternatives, and designates the origin from a depth of one, two, or three dermatogen cells as types A, B, and C, respectively. This, however, seems to be laboring a rather simple matter, since the number of cells involved will probably largely depend on their actual dimensions at the point where the future primordium is to arise. For example, two daughter cells resulting from a recent anticlinal division would probably both divide periclinally, whereas a large dermatogen cell might tend to divide periclinally first and then the two daughter cells divide anticlinally.

Although the first dermatogen cell to divide periclinally may not be situated at the center of insertion of the future primordium, and even the first two cells to divide may be far apart on each side of the future middle line, later activity soon produces a sector of periclinally dividing cells, with divisions most rapid in the middle of the primordium (figs. 5B, C, D; 7B, C). This would suggest that some internal stabilizing influence is at work insuring that the new primordium continues the strictly distichous arrangement of the leaves.

ENCIRCLING GROWTH OF PRIMORDIUM.

—The rapid lateral spread of the periclinal type of division in the dermatogen and hypodermis is responsible for the transformation of the original rather

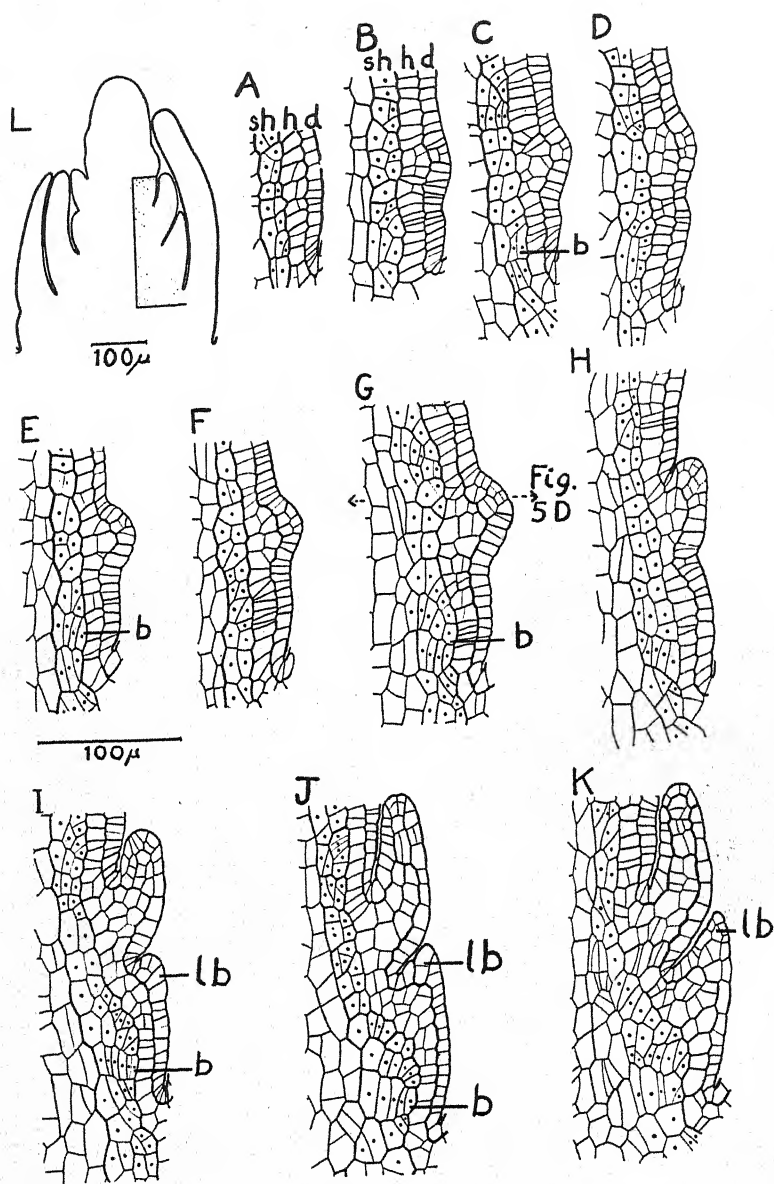


FIG. 4.—Radial longisections in plane of leaves showing stages in initiation of primordium on side of apex. *b*, cells which foreshadow bud in axil of leaf lower down; *lb*, base of primordium growing around from other side of axis. *d*, *h*, *sh*, dermatogen, hypodermal and subhypodermal layers. (Dots employed to indicate subhypodermal layer are purely conventional and in later stages are not intended to define absolutely the inner limits of this layer.) *L*, longisection of whole apex showing position and extent of tissue included in *K*.

local protuberance into a crescent and then into a collar. Because the leaves are in two ranks, in radial longitudinal sections the encircling growth of those on one side of the apex begins to show in the positions alternating between the insertions of the primordia on the other side (figs. 2, 3). In figures 4I and J, for example, the conditions of the cells immediately above and below the insertion of the primordium represent the stages reached by the encircling growth of the leaves inserted on the other side of the axis above and below the primordium actually figured. Thus, for convenience of illustration, for each primordium only it itself plus a small portion of the axis above and below are shown, rather than the whole section actually concerned—or the “disc of insertion” as BUGNON would call it. The portion illustrated is indicated in figure 4L, and reference to the longitudinal sections of complete apices in figures 2 and 3 may help in this matter.

The periclinal divisions in the cells of the hypodermal layer in a position on the axis below the primordium in figure 4D and the divisions above and below in figure 4E indicate the spreading-around of the insertion of the leaves on the other side of the axis. Whether the first indication of this spreading comes early (as in fig. 4E) or late (as in fig. 4H) depends on the actual apex concerned. Usually, some time after the hypodermal cells have divided periclinally the dermatogen cells do the same (figs. 4H, I, J; 6E, F, G), so that the two layers become responsible for the tissues of the up-pushing which marks the encircling base of the leaf insertion on the other side of the axis. This is clearly seen in the case of primordium 11 in figure 3.

In the apices of rhizomes and young aerial shoots the two encircling edges of

the primordium meet at the same level; but in older shoots apparently the two sides overlap, giving the appearance shown for primordium 9' in figure 3. This seems to be accomplished on one side by the outer wing continuing its encircling growth by dermatogen (and hypodermal?) divisions on the outer face of the future inner wing, and presumably the inner wing doing the same by divisions on the inner face of the outer wing on the other side of the mid-line. Thus the future sheath is tubular at the extreme base but has two overlapping edges higher up. No doubt, differences in the rate of encircling growth cause differences in the degree to which the sheaths appear “split” or “fused” in various grass species (see ARBER [2], quoting for *Festuca* from HACKEL). It is often difficult to picture exactly what happens during the development of the overlapping portions, but some similar course as this probably explains the condition in *Zea*, where the overlap at the insertion may be as much as half the circumference of the stem.

There seems little doubt that the hypodermal cells contribute to the tissues of the lower part of the primordium, as suggested by BUGNON and KLIEM. The primordia shown in figures 4K and 6E, F seem to permit no other explanation. Cleared apices also suggest that the primordia are not derived solely from the dermatogen, as reported by DOULIOT (12), whose work was confined to hand sections, and by RÖSLER (18), who conceived that the very young primordium (*Blatthöcker*) arose from the dermatogen alone but seems to have left open the question of its later development.

During the early growth of the primordium the cells throughout divide actively, and rapid increase in its length is due to the fact that most of the divisions are at right angles to the long axis,

giving files of cells in the interior and at the surface of the leaf. This is a striking feature when grass shoots are being dissected under a binocular microscope, the shining facets formed by the convex outer walls of the epidermal cells forming lines of bright dots. Occasional longitudinal divisions contribute to the width of the leaf, causing the appearance of two files of cells in continuity with only one at the top and bottom. Occasional longitudinal divisions parallel to the future upper and lower surfaces lead to the increasing thickness of the young leaf.

The cells of the lower (abaxial) surface divide more rapidly and at the same

and the original limits between the cells derived from the dermatogen and hypodermis are much lower, probably about half-way down the primordium.

At the same time, the cells at the margins of the primordium are also adding to the internal tissues at the wings and appear as two continuous bands running from the tip to the insertion. They are behaving in the same way as the initials at the tip and are not really separable from them, as the whole forms what is originally the free edge of the ring or collar and later the tip and sides of the cowl as the primordium changes its shape. This is very striking in cleared material.

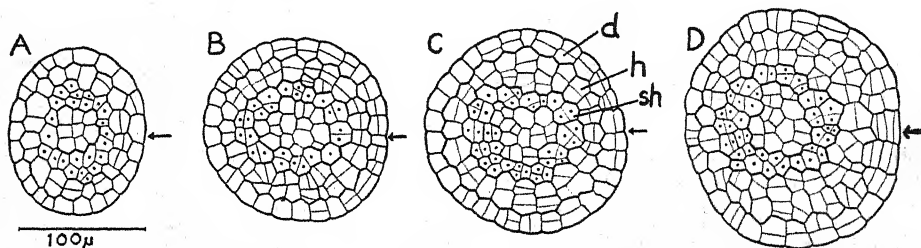


FIG. 5.—Transsections showing stages in initiation of primordium (arrows indicate mid-line of future leaf). D is taken at about level indicated in fig. 4C. *d*, *h*, and *sh*, dermatogen, hypodermis, and subhypodermis. Dots used to indicate subhypodermal layer are conventional and not intended to imply any noticeable difference in contents.

time become more elongated than those on the inner surface, with the result that the primordium assumes a vertical position (fig. 4G–J) and so becomes converted from a ring into a small collar (fig. 1).

For a considerable time, usually until the primordium is about to overtop the apex, new tissue is being added to the interior by the cells at the free edge and tip. This is indicated by the periclinal divisions in the cells shown at the tip of the primordia in figure 4H, I, J, and in the ninth and eleventh primordia of figure 3. Thus the whole of the small mass of tissue illustrated in these figures has been derived lately by apical activity,

When the young leaf is beginning to inclose the younger primordia and the apex, the cells in the position of the future midvein start to divide longitudinally, the median provascular strand is formed in the leaf and axis, and then the primordium rapidly overtops the apex and begins to leave the apical region. At about this time the ligule arises by periclinal divisions in two or three horizontally running rows of cells of the epidermis of the adaxial surface, exactly as described for *Zea* (21, 22).

ORIGIN OF AXILLARY BUDS.—So far little mention has been made of the axillary buds, although their first stages are evident very early. When periclinal di-

visions in the dermatogen and hypodermal layers mark the initiation of a new primordium, the cells of the underlying subhypodermal layer themselves divide once periclinally and usually only rarely divide in that plane again until the primordium has become a distinct collar (figs. 3; 4*A-I*). However, they do divide actively just above the primordium, that is, in a position just below the encircling insertion spreading around from the leaf situated above on the opposite side of the axis. They are marked *b* in the figures.

The more central cells of this new activity in the subhypodermis divide periclinally, but those immediately above and below divide by walls which are curiously half periclinal and half anticlinal (fig. 4*G*). This results in a tissue composed of cells with walls which all appear to be on the circumferences of circles whose center is the position of the future bud (figs. 4*C, E, I, J*; 6*D, F, G* at *b*). It seems that the tissue is derived from a vertical depth of four or five cells, although the more central two or three are the most active.

In transverse section this tissue appears as a few radially running files of cells (fig. 7*D-F*). Figure 8*A* represents a transverse section of about the stage seen in the lower part, at the level *b* in figure 4*A*. Figure 8*B, C*, and *D* show stages comparable with those seen in the lower part of figure 4*C, D*, and *E*, respectively, while figure 8 *E-G* represent a close series of stages at about the maturity shown in figure 4*G-J*. Figure 6*H* is probably comparable with figure 4*K* or figure 9*A* or *B*.

To link up these sections of bud stages with those illustrating the initiation of the leaf primordium, figure 8*A* can be regarded as about the condition to be found in sections immediately below the

one illustrated in figure 5*A*. Figure 8*B* or *C* would come under that shown in figure 5*B*; that of figure 8*D* would come under that of figure 5*C*; and the stages in figure 8*E* or *F* would probably be found under such a stage as that shown in figure 5*D*.

In transverse sections in the region of the future bud, the cells of the hypodermis are often seen to be divided half anticlinally and half periclinally, so that the planes of their new walls (in cells *x* in figure 8*C* and *D*) are more or less parallel to those of the files of cells in the subhypodermal layer. It is rather as though the divisions were spreading across from the subhypodermal layer into the hypodermis at the sides. The cells of the hypodermis a little nearer the center of the future bud tend to divide (fig. 8*D, y*), and emphasize the rather bowl-shaped appearance of the tissue in this region. At the center there seems to be one or more hypodermal cells which divide only periclinally (figs. 4*I, J*; 7*G-I*; 8*E-H*; 9*A*). Presumably these are destined to be the initials of the hypodermis of the future bud apex.

In figure 8*G* and *H* only the more central cells intimately connected with the bud are dotted; their inner limits are not suggested as they are difficult to determine. Although in the region of the future bud it is the subhypodermal cells which divide most actively, at the sides of the axis the hypodermal cells are the most active (fig. 8*D-F*). Thus although the inside of the subhypodermis is more or less circular in cross-section, the outer limits are oval. In figure 8*G* and *H* the tissue on each flank is undoubtedly derived from the hypodermis, although it is not certain to what depth it extends in these stages. This lateral activity of the hypodermis is also visible at the level of the middle of the primordium (fig. 5*B, C*).

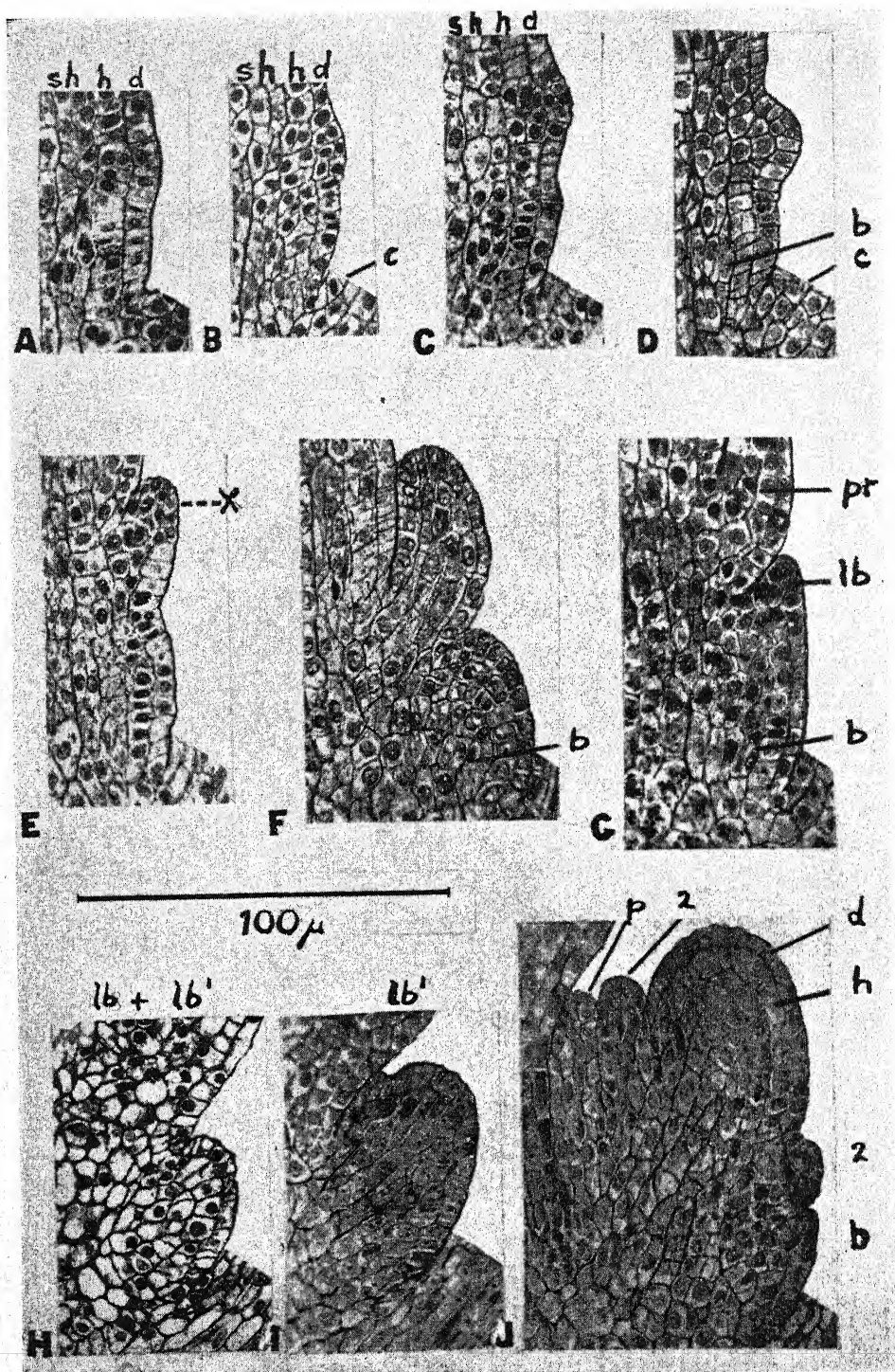


FIG. 6.—Radial long sections. *A–F*, in plane of leaves, showing initiation of primordium on side of apex. *d*, *h*, *sh*, dermatogen, hypodermis, and subhypodermis. *c*, base of leaf (removed) inserted directly below; *b*, cells which foreshadow future bud. *G*, section through base of primordium (*pr*) and encircling insertion (*lb*) of next oldest leaf growing around from other side of axis. *H–J*, sections in plane of leaves of main axis showing development of axillary bud. *d*, *h*, dermatogen and hypodermal layers; *p*, prophyll; *2*, second leaf of bud; *lb*, *lb'*, two overlapping wings growing around from primordium above on other side of apex.

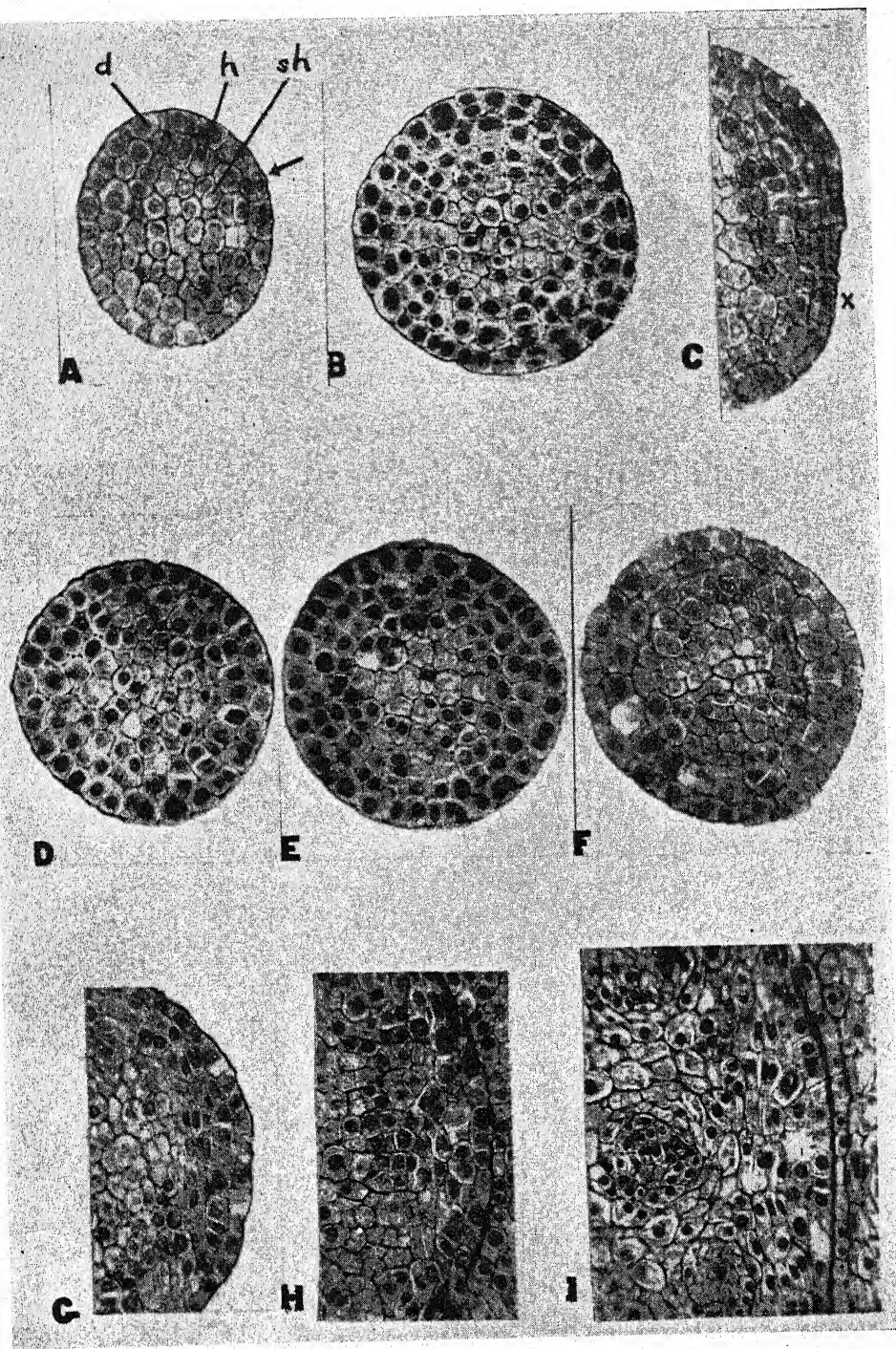


FIG. 7.—Transections of axis. *A–C*, at point of initiation of leaf primordium. (Arrow in *A* indicates first periclinal division which could be found connected with future primordium, although in this and other sections a number of hypodermal cells have already divided periclinally.) *C*, taken at level (*x*) shown in fig. 6*E*, showing further divisions of dermatogen cells (at *x* it would seem that one of the inner daughter cells divided anticlinally before dividing again periclinally). *d*, *h*, *sh*, dermatogen, hypodermis, and subhypodermis. *D–I*, stages preceding development of axillary bud: *D* at about same stage as that in fig. 6*C*; *E* and *F* at about that in fig. 6*D*; and *G–I* between that of fig. 6*G* and 6*H*.

At about the stages represented in figures 4*J* and *K*; 8*F* and *G*; and 9*A*, the cells at the outside of the central files seem to have divided more actively and are often smaller, with rather more densely staining contents. They are presumably the future initials of the subhypodermis and core of the bud axis.

to be situated. This view is also supplemented by the way in which its subsequent behavior is correlated with events in the leaf above rather than in the one below (22).

EMERGENCE OF AXILLARY BUD.—Figures 6*G*–*J* and 9*A*–*I* are of radial longitudinal sections in the plane of the

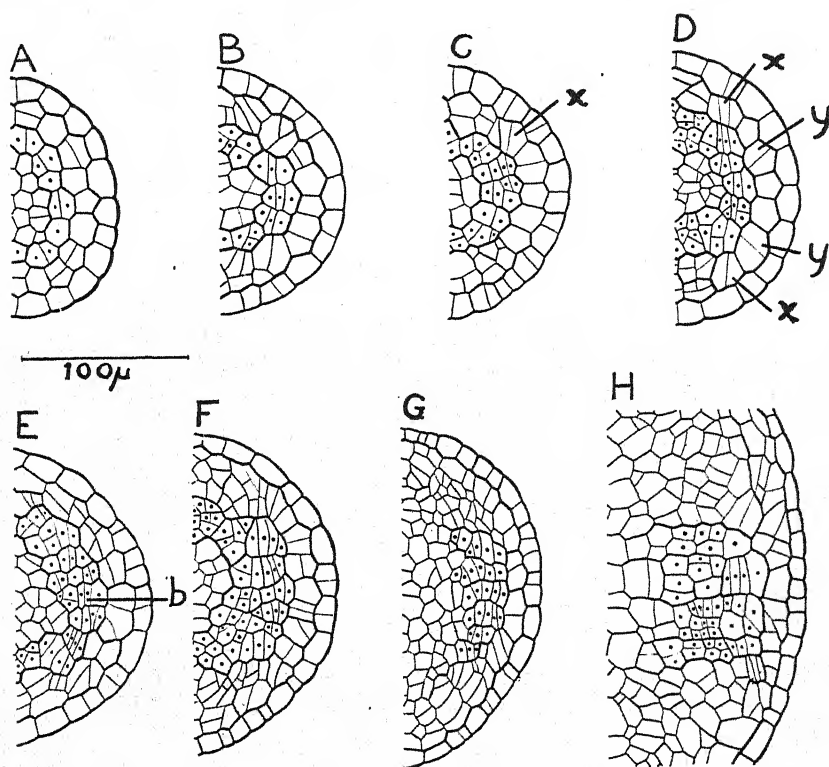


FIG. 8.—Transsections showing preceding stages in development of axillary bud. *b*, files of cells shown in longisection in fig. 4. Dots used to indicate subhypodermal layer are conventional and the limits of only part of this layer are suggested in *G* and *H*. *x*, *y*, divisions in hypodermal cells referred to in text.

The position of the files of cells foreshadowing the axillary bud is interesting because of the manner in which it is so intimately bound up with the development of the encircling growth of the leaf above and on the other side of the axis. This suggests that the bud should be associated with that leaf rather than with the one in whose axil it will later appear

leaves of the main axis and show stages in the emergence of the axillary bud. It will be seen that at the beginning a vertical depth of four or five files of subhypodermal cells (dotted) is involved, but as development proceeds the tissue of the inner part tends to be confined to about the middle three files—about nine to twelve cells in cross-section of the new

bud axis. The cells derived from these files undergo further divisions, so that there appear to be up to about six files at the base of the bud in longitudinal section (fig. 9*F-H*). In figure 9*A-C* the

The tissues just behind the bud are penetrated by the median provascular strand to the leaf but one above (to the next leaf inserted on this side); such a stage is shown in figures 7*I* and 9*C*.

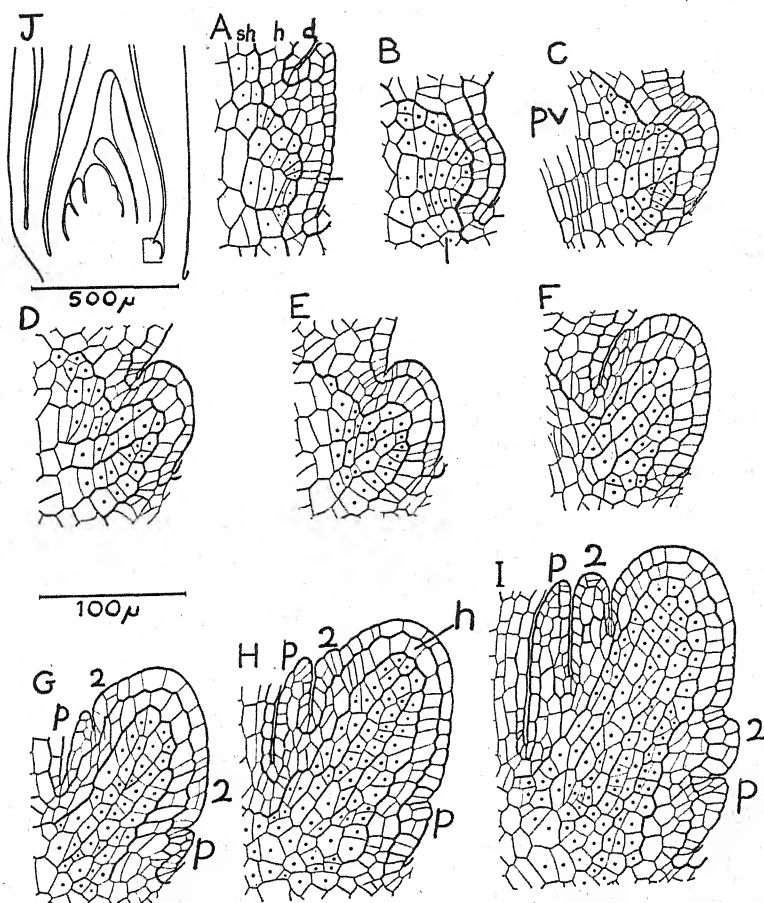


FIG. 9.—Radial longisections in plane of leaves on main axis showing development of axillary bud. *d*, *h*, *sh*, dermatogen, hypodermis, and subhypodermis. *lb*, encircling base of leaf above and on other side of axis. *p*, prophyll of bud; *2*, second leaf of bud; *pv*, provascular strand in main axis. Dots in subhypodermal layer are not intended to define inner limits of this layer. *J*, longisection of whole apex showing orientation of tissue drawn in *E*.

hypodermis above the bud has increased in width where the insertion is coming around from the leaf above; figure 9*J*, which shows the position of the bud in figure 9*E*, helps to interpret the relationships of the structures at this stage.

Lateral provascular strands to the insertion of the primordium of the leaf above (inserted on the other side of the axis) are differentiating out of the tissue at this stage. Possibly this differentiation of provascular strands in the "parent"

axis is correlated with emergence of the bud.

The bud begins to grow outward mainly by the activity of the subhypodermal cells. At the same time it takes a course upward, so that its own axis is at an angle of about 45° with that of the "parent." This is brought about by the more rapid divisions in the files of subhypodermal cells on the lower side. About the time the bud begins to grow out, periclinal divisions take place in the hypodermis on the upper side of the bud (fig. 9E), followed by divisions in the dermatogen (fig. 9F), indicating the initiation of the first leaf of the bud—the prophyll. Sooner or later other traces of it may be seen below the bud, as in the divisions of the hypodermal cells in figure 9F and those in the hypodermal and dermatogen cells in figure 9G, H. Later, as at "2" in figure 9G, H, and I, parts of the second bud leaf appear. In the case of the leaves on the bud, the parts appearing on each side of the axis seen in these sections are the insertions spreading around the bud axis. A longitudinal radial section of the main axis in the plane of the leaves cuts the bud axis at right angles to the plane of its leaves. Thus a section of a bud which is fairly well grown out, as in figure 9I, shows the conditions obtaining at right angles to that illustrated in figures 2 and 3. Of course, it is rarely possible to go exactly vertically down the whole bud axis, hence some of the files seen in figure 9I are not complete from the base to the top.

No periclinal divisions are found in the dermatogen at the position of the tip of the future bud, neither during the early stages of the initiation nor at any subsequent time during its emergence (figs. 6G-J; 9A-C). Although periclinal divisions play their part in the produc-

tion of the prophyll (fig. 9F), the dermatogen contributes nothing to the internal tissues of the axillary bud apex.

As is evident from the figures, the divisions in the hypodermal layer at the tip of the bud have all been anticlinal. Careful examination of numerous series showed—provided one was dealing with the center of the bud—no periclinal divisions. Of course, sections off the median showed such divisions in the hypodermis *apparently* at the tip, but in such cases one is looking at the divisions in the position of the next primordium.

RÖSLER (18) and KLIEM (15) stated that, in *Triticum* and *Avena*, respectively, the new bud apex grows by only two sets of initials, one for the dermatogen (tunica) and the other for the internal tissues (corpus), thus following the mode of growth of the parental apex. RÖSLER figures periclinal divisions in the cells of the hypodermis at the tip of the emerging bud apex about the time the prophyll is being initiated a little farther back (18, fig. 15g and h and plate IV, fig. 39). This would mean that although the "core-plus-subhypodermis" of the new bud starts by being derived from the subhypodermis of the parent, it is later extended by tissue being cut off from the hypodermal initial or initial group. Assuming that this appearance is real and not caused by the examination of sections slightly off the median, it may well be that in both *Triticum* and *Avena* the bud apices resemble the main shoot in having less massive apices than *Agropyron* and consequently a more "juvenile" type of growth. In RÖSLER's figure 39 the bud apex has a width of six cells, which would mean about twenty-seven cells in a transverse section of its axis. In the comparable apex of *Agropyron* (fig. 9H or I) there are eight or nine cells in

its width, making about fifty to sixty cells in cross-section.

This difference in size of the emerging apex seems to be supported by differences in the early stages in the subhypodermis. In *Triticum* and *Avena* a vertical depth of only about three subhypodermal cells (RÖSLER fig. 15a-d; KLIEM fig. 30) and in *Triticum* a tangential width of about two to three cells foreshadow the future bud. When the bud actually begins to emerge, in *Triticum* (and possibly in *Avena*) the derivatives of a vertical depth of only two cells often play a part in the formation of the early core. In *Agropyron*, on the other hand, a vertical depth of four to five cells (figs. 4I-K; 9A, B) and a tangential width of three to four cells (fig. 8H) foreshadow the future bud. In the core of the emerging bud the derivatives of a vertical depth of three to four cells are actually represented (fig. 9D-G). Thus in the solid, in *Triticum* (and presumably in *Avena*) the early core-plus-subhypodermis of the bud is composed of files of cells derived from about four to six of the original subhypodermal cells, and in *Agropyron* it is made up of nine to twelve similar files.

From about the stage shown in figure 9H, the center of the bud axis begins to develop as a distinct core (fig. 9I). The central tissue originally derived from the subhypodermal layer of the parent axis is being delimited into an outer shell (the new subhypodermis of the bud) and an inner core two or three cells in diameter at the top. Thus the new core of the bud is not derived from the core of the main axis but from the "parental" subhypodermal layer. At the stage represented in figure 9I the apex appears to be growing in the same way as the parent: separate initials or initial groups are probably present for (a) the dermatogen, (b) the hypodermal layer, and (c) the

new subhypodermal layer and the core within.

Deductions and inferences

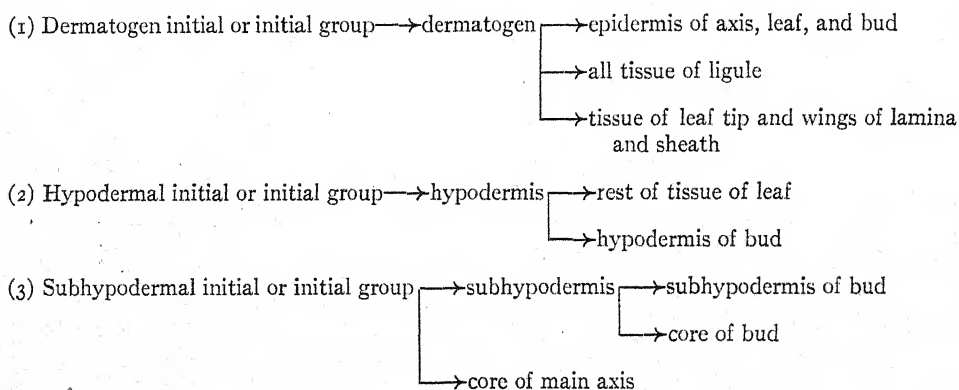
APEX, LEAF, AND BUD PRIMORDIA.—In all of more than twenty species of grasses which the writer has examined, the conditions are substantially as those described for *Agropyron*. Always the dermatogen and the hypodermis have been concerned in leaf production and the subhypodermal layer called into play at bud formation. This is not taken to mean that these three layers have any phylogenetic importance, or that they are to be considered too rigidly significant. It is conceivable, for instance, that in some particular species the leaves may be derived solely from the dermatogen, or in some other "atypical" way. The real significance would appear to be that the normal leaf is of relatively superficial origin, while the axillary bud incorporates deeper tissue. The summary on page 286 of the mode of leaf and bud initiation in *Agropyron* probably holds good for the majority of the Gramineae, and, moreover, is probably correct for the modified leaves like glumes, etc., as well.

In a young grass shoot, or where the apex is less massive, there may be only one common initial or initial group for both (2) and (3), but periclinal divisions in the cells cut off result in the production of a hypodermis and a subhypodermis which behave in the same way as above.

APPLICATION TO GRASS AND CEREAL BREEDING.—The mode of origin of the buds has a special significance in cereal and grass breeding techniques involving the use of colchicine to obtain amphidiploids. In the Gramineae the ovule is not derived from the carpel wall but from the residue of the axis on which the palea,

stamens, carpel, etc., are borne. The archesporial cell appears in the hypodermis and is apparently usually the initial cell of this layer; it gives the megaspore mother cell without further division. In the case of the stamen the situation is not so clear, for although the archesporial tissue is derived from the hypodermis of the young anther, it is not at all certain whether the anther itself is initiated as a normal leaf involving periclinal divisions in the dermatogen cells, or whether it is more in the nature of a bud

the plane of the leaves—all the buds on that side will have their hypodermis with the double number of chromosomes and will give viable gametes. The question of whether the flowers are borne on a tertiary or a quaternary branch or on one of any higher order has no significance. The younger the seedling when treated, the better, first because there are less initials to the hypodermis in young apices (RÖSLER and KLIEM), but mainly because young buds grow out into whole shoots and later inflorescences,



—a situation which appears very likely, judging from its morphology in dissected material and the illustrations of similar material which have been published (6-10, 16). A preliminary study of *Anthoxanthum* seems to indicate that it is budlike and that periclinal divisions do not add to its inner tissues. A similar origin has been reported for the stamens of *Datura* (19).

Thus, if in a sterile hybrid viable pollen and ova are to be produced, the cells which must be made amphidiploid are those of the hypodermis. If at an early stage in the development of the seedling one of the hypodermal initials has its chromosome complement doubled, a sectorial chimaera will be produced, and then—supposing the doubled sector is in

while higher buds give a whorl of branches in the case of a panicle or a single spikelet in the case of a spike. Doubling the number of chromosomes in one or more hypodermal cells on the side of the apex will be effective in producing only one bud or a block of buds with the double complement.

As far as the breeder is concerned, it is of no consequence whether the hypodermis of the main shoot of his particular hybrid is produced by an initial or initial group of its own in the manner which seems to be followed in *Agropyron*, or whether it shares a common initial or initial group with the subhypodermis and core, which according to RÖSLER and KLIEM is effectively the case during the greater part of the life of *Triticum* and

Avena. Where the hypodermis does share the same initial or initial group with the subhypodermis and core, then doubling the number of chromosomes will cause all or a sector of the core cells to have the double number as well as the hypodermis. This may be an advantage for the future healthy development of the shoot, but it obviously does not affect the theoretical side of the question of the constitution of the axillary buds. Similarly, from the plant breeder's point of view, it does not matter whether the axillary bud grows with a common initial for the internal tissues in the manner suggested by RÖSLER and KLIEM for *Triticum* and *Avena*, or by separate initials for the hypodermis and subhypodermis-plus-core as described here for *Agropyron*, since in both cases the new hypodermis—the part which is important—has been derived directly from the hypodermis of the parent axis.

Doubling in the dermatogen will be useless as far as the axillary buds are concerned, but may perhaps be of some use if a considerable amount of the inner tissue of the stamen tips should be derived from it. Doubling of any initial or any of the cells in the subhypodermis or core will be useless unless some unusual development occurs later.

Discussion

In this study the description has been made purely objective, with the intention of avoiding all formal histogenic viewpoints and of avoiding also the introduction of theories about a determinate zonation of the apical tissues, either of the HANSTEIN or the BRESLAU types. Should it be argued that this is a sterilized outlook and that much is to be lost by the method, it may be defended that a formalized viewpoint is likely to obscure more facts than it reveals. To the

writer the initiation of a primordium or a bud is not due to any inherent properties or "prospective values" of any layer, nor is it due to the cells concerned being derived from any special initial or initial group, but rather to a change in the type of metabolism of the particular cells involved; that is, an increase in their rate of protoplasm synthesis, rate and direction of division, etc. Since all the apical cells are fundamentally omnipotential, this change must be regarded as due to a change in their environment; that is, to a change in the direction, type, or speed of entry of "food," aeration, etc. The difference between the cells of the dermatogen and of the hypodermis, for example, is not inherent in the cells themselves, or due to their ultimate origin, but to what might be termed their position-metabolism. How the effects of differences in availability of "food" or water, changes in acidity or aeration, etc., are integrated is as yet totally unexplored; in fact, we do not even know whether a cell divides parallel to or at right angles to the resultant of all these factors. For instance, when the dermatogen cells begin to divide periclinally, has there been a swing around in the direction of the resultant of the influences at work on the particular cells?

The various layers of cells in the apex are to be likened to a gardener's topsoil and subsoil—differences in types of activity and not in original constituents. Rigid use of the HANSTEIN type of zonation, or even of the more elastic tunica and corpus scheme, will only help to cloud over the many difficulties underlying the true picture of the processes at work during the initiation of the organs. (It might be added that FOSTER [13, 14] has expressed similar views.) If the more fluid "metabolic" type of zonation is substituted, then—although the

depth of the various layers may be fairly constant for related plants—variation would be expected if a wide series of types were examined: hence the layers of two, three, four, etc., cells reported for the “periblem,” or the layers of one, two, three, etc., cells called the tunica by others; hence also the difference in the number of these layers between the main and axillary shoots of the same species (25).

Again, this idea of a nonrigid “metabolic” differentiation of the apical cells makes intelligible the occasional occurrence of periclinal divisions in the dermatogen, independent of the production of any future primordium, as reported elsewhere for *Triticum* (18) and *Zea* and *Agropyron* (20, 24).²

Summary

1. The rhizome and early aerial shoot of *Agropyron repens* Beauv. each possesses a relatively short apex bearing only a few primordia. During the growing season the apex becomes more elongated and bears more primordia, which now show smaller steps in advancement. Later the apex elongates considerably and becomes transformed into the inflorescence. Of the axillary buds which grow out, the highest ones produce “blind” shoots, the lowest develop into shoots ending in inflorescences, and those underground give new rhizomes.

2. The shoot apex consists of three thimble-shaped layers—the dermatogen, the hypodermis, and the subhypodermis, which inclose a central core. There are probably three separate sets of initials or initial groups, one each for the

dermatogen and the hypodermis and a common one for the subhypodermis and core.

3. Leaf primordia can first be detected by isolated periclinal divisions in the dermatogen cells on the side of the apex. These divisions are themselves probably preceded by similar ones in the hypodermis. Further divisions in the dermatogen and the hypodermal cells lead to the production of a crescentic protuberance. Increased activity in the region of the future mid-insertion insures the continued distichous phyllotaxis of the shoot. Lateral spreading of the divisions around the axis converts the crescent into a collar which then grows upward and produces a miniature cowl inclosing the apex and the younger primordia which have since arisen. Continued activity of the dermatogen at the free edge of the collar and then the cowl adds tissue to the tip and margins of the primordium. The internal tissue of the young leaf is derived from both the dermatogen and the hypodermis; the subhypodermis and core contribute nothing.

4. The axillary buds, which are foreshadowed by the production of radial files of cells in the subhypodermis, emerge soon after the insertion of the leaf above has spread around from the other side of the apex. No periclinal divisions occur in either the dermatogen or the hypodermis at the position of the future bud tip, so that these two layers in the bud are derived directly from the same layers in the main shoot. The subhypodermis and core of the bud are derived from the subhypodermis of the main axis.

5. The mode of origin and development of the axillary buds should be borne in mind when amphidiploids are to be produced by the action of colchicine on seedlings or young plants.

² Also, in a letter dated 10/3/41, Dr. A. S. FOSTER wrote: “One of my graduate students, working on *Triticum*, has found that periclinal divisions occur in the surface cells at the summit of the apices of seedling and young axillary buds.”

6. It is suggested that the zonation observed in the apex is not due to any particular qualities inherent in the cells themselves but to the particular types of metabolism in these strata. This leads

to a less rigid concept than that of either the HANSTEIN or the BRESLAU schools.

BOTANY DEPARTMENT
THE UNIVERSITY
LEEDS 2, ENGLAND

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DEVELOPMENT OF REPRODUCTIVE STRUCTURES IN THE BROWN ALGA *TURBINARIA TURBINATA*

H. L. BLOMQUIST

Introduction

Prior to the work of SIMONS (23) on *Sargassum filipendula* C. Ag., not much was known about the reproductive structures of the phaeophycean algae now included in the family Sargassaceae. SIMONS made two important contributions. First, she traced the origin of the cryptostomata and conceptacles to an epidermal initial cell, which in its first division by a downwardly curved, transverse wall gives rise to an outer, narrow cell, which she called the "tongue cell," and to a larger "inner cell." The latter she found to be the origin of the cells lining the conceptacle, which eventually bear the paraphyses, antheridia, and oogonia. Second, she observed a one-celled oogonium which was assumed to function directly as the egg without undergoing reduction division.

To check these observations, NIENBURG (19) investigated the conceptacle development of *Cystoseira barbata* (Godd. and Woodw.) C. Ag., and other genera of the order Fucales, and the oogonial development of *Sargassum linifolium* (Turn.) C. Ag. (18). NIENBURG agreed with SIMONS as to the origin and development of conceptacles in the representatives of the Sargassaceae which he studied, but he concluded that meiosis in *Sargassum* takes place in the oogonium and that this occurs after the oogonium is discharged and while it remains attached by a mucilaginous strand to the inside of the conceptacle. He also noted that meiosis is followed by another nuclear division resulting in eight nuclei, seven of which disintegrate within the cytoplasm of the egg. He found

the development in *C. barbata* somewhat different, in that the oogonium remains *in situ* during meiosis and the seven supernumerary nuclei are extruded from the cytoplasm.

Since the completion of these investigations, considerable work has been done on other members of the Sargassaceae, especially by English, New Zealand, and Japanese investigators. Included in these studies are the genera *Bifurcaria*, *Carpophyllum*, *Coccophora*, *Cystophyllum*, *Cystoseira*, *Sargassum*, and *Turbinaria*; and the features which have received attention are: conceptacle development, oogonial maturation and discharge, cytology, and embryology.

While serving as an exchange professor at the University of Puerto Rico during the academic year of 1941-1942, an opportunity was afforded me for collecting material of the genus *Turbinaria*. This led to a study of the reproductive structures of this genus, which has received limited attention in previous studies.

In Puerto Rico, *Turbinaria* occurs most frequently on the northern or Atlantic side, where it grows on rocky substrata in two distinct locations. One is the eroded depressions in consolidated sandstone outcrops at or slightly above the mid-tide level, where the breakers strike the rocks with considerable force so that the spray or waves not only keep the depressions filled but by overflow maintain a constant supply of fresh water. Because of the low tides which prevail in the West Indies, these depressions are not often completely submerged, at least not to any considerable depth. In

such places the plants are rather small. Other algae associated with *Turbinaria* in these habitats are *Digenea simplex* (Wulfen) C. Ag., *Anadyomene stellata* (Wulfen) C. Ag., *Dictyosphaera favulosa* (C. Ag.) Decaisne, *Valonia ocellata* Howe, *Sargassum lendigerum* (L.) Kütz., etc. Other locations are in deeper water (3–5 feet) in more sheltered situations. Here the alga grows to larger sizes; one plant collected measured nearly 3 feet in length and weighed approximately 10 pounds. In these habitats it is usually associated with *Sargassum polyceratum* Mont., *S. platycarpum* Mont., *Bryothamnion triquetrum* (Gmel.) Howe, *Penicillus capitatus* Lam., species of *Galaxaura*, etc. To what extent it occurs at greater depths was not determined; but since it is not found attached in abundance in accessible places and yet whole plants and fragments are constantly being washed ashore, one may conclude that it occurs in considerable amount in deeper water.

The early taxonomic history of *Turbinaria* was reviewed by BARTON (2). According to her systematic treatment, eight species are recognized. DE TONI (8) listed nine species, while KJELLMAN (15) estimated three to five. On the basis of its general morphological features, it was early considered to be closely related to *Sargassum*; and in the establishment of the Sargassaceae, DE TONI placed it in this family.

According to BARTON, two species of *Turbinaria* occur in the West Indies. The most common of these is *T. turbinata* (L.) Kütz. The other, *T. tricostata* Barton, seems to be rare and may not differ sufficiently from the former to deserve specific rank (10). All the material used in this study has been referred to *T. turbinata*.

Material for imbedding in paraffin

was fixed in chromic-acetic, formalin-acetic, or simply formalin. Plants were also pressed and dried for herbarium specimens.

The only morphological work dealing with *T. turbinata* was done by BARTON (2). Other species which have received attention by Japanese investigators are *T. fusiformis* Yendo (28), *T. ornata* J. Ag., and *T. filiformis* (12, 13, 14).

Observations

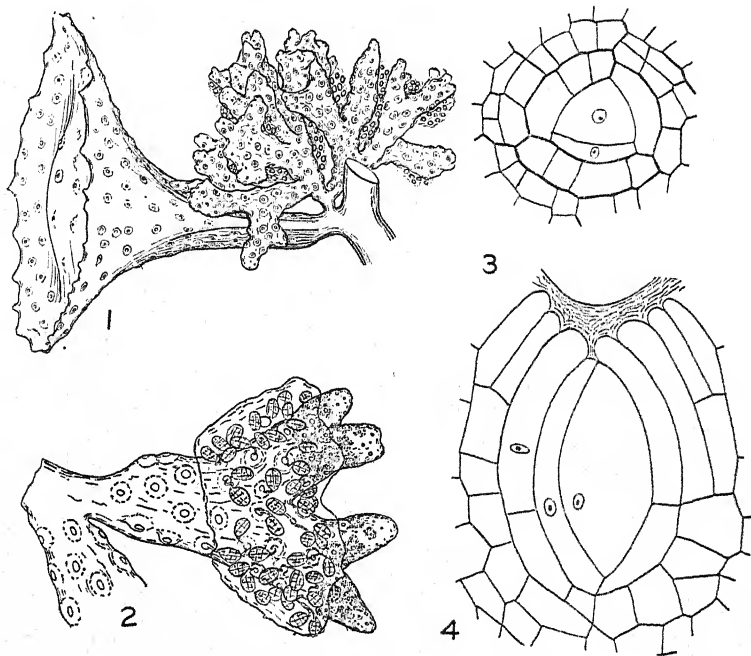
RECEPTACLES

The receptacles in *Turbinaria*, as in *Sargassum*, *Landsburgia*, and *Carpophyllum*, are axillary to the "leaves" or "phylloclads." They are richly branched, forming an irregular corymbose cluster (fig. 1). As was noted by BARTON, growth is initiated by a sunken apical cell which is triangular in cross-section (fig. 3). This type of initial cell is characteristic of the Sargassaceae. An exception seems to be *Carpophyllum flexuosum* (Esp.) Grev., in which DAWSON (3) found it to be truncated lenticular—as in *Fucus*. As to the shape of this apical cell longitudinally, there has been some uncertainty owing to the difficulty of obtaining strictly median sections or of knowing just when this was accomplished. After a careful study of transverse and longitudinal sections, it is concluded that in *T. turbinata* it is lenticular but may vary somewhat in symmetry (fig. 4).

In the branching of the receptacle, new apical cells originate from a preceding one, so that the tip of a receptacle shows a number of apical cells around a central one. As these are apparently formed in spiral succession, branching is fundamentally spiral, but this arrangement is more or less obliterated by irregularities in growth.

The genus *Turbinaria* has generally been considered strictly dioecious. While this may be true of *T. fusiformis* (28), *T. ornata*, and *T. filiformis* (13), a different situation is found in *T. turbinata*. Although plants are commonly of one sex, this species not infrequently shows hermaphroditic conceptacles. Also, occasionally both antheridial and oogonial con-

ceptacles appear in one receptacle. BAR-
TON observed hermaphroditic concep-
tacles also in *T. conoides* Kütz.



FIGS. 1-4.—Fig. 1, "leaf" with axillary receptacles. Fig. 2, receptacle with discharged oogonia in embryological development. Fig. 3, receptacle initial cell in transection. Fig. 4, receptacle initial in longisection.

ceptacles appear in one receptacle. BAR-
TON observed hermaphroditic concep-
tacles also in *T. conoides* Kütz.

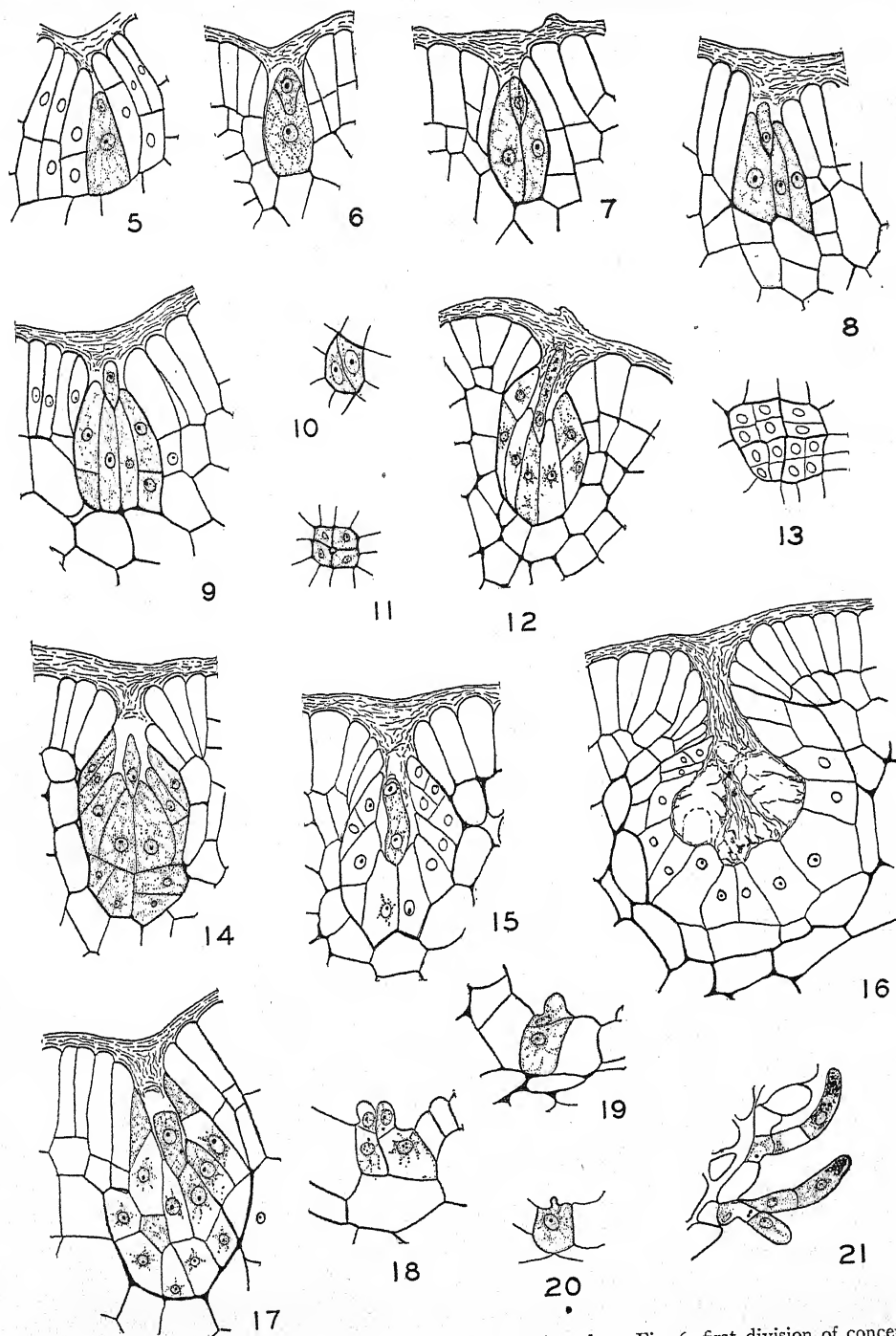
CONCEPTACLES

Conceptacles originate from an epi-
dermal cell which in longitudinal section
is first distinguishable near a receptacle
initial. It differs from the surrounding
epidermal cells in its slightly depressed
position, owing to the delayed first trans-
verse division, and in its Erlenmeyer-

served by DAWSON (3) in *Carpophyllum*
flexuosum.

The first division of the conceptacle
initial is transverse by a downwardly
curved wall (fig. 6), as in *Sargassum* and
other genera of the Sargassaceae in which
it has been observed.

The outer of the resulting two cells,
the tongue cell, may develop into a pa-
raphysis or may sooner or later disinte-
grate. In antheridial conceptacles the
tongue cell usually develops into a nor-



FIGS. 5-21.—Fig. 5, conceptacle initial cell with divided nucleus. Fig. 6, first division of conceptacle initial forming tongue cell and inner or basal cell. Fig. 7, first division of basal cell. Figs. 8, 9, further divisions. Fig. 10, first division in transverse view. Fig. 11, second division in transverse view. Fig. 12, young oogonial conceptacle with degenerating tongue cell. Fig. 13, transverse view of stage of conceptacle shown in fig. 12. Fig. 14, young oogonial conceptacle with undivided tongue cell. Fig. 15, with two-nucleate tongue cell. Fig. 16, with disintegrating tongue cell forming mucilage. Fig. 17, young oogonial tongue cell undivided. Fig. 18, paraphysis initial cell with papilla. Fig. 19, papilla cut off from paraphysis initial. Fig. 20, two papilla developing adjacently. Fig. 21, "periphyses" below rim of ostiole.

mal paraphysis, which, owing to its early start, may be distinguished for some time from the surrounding paraphyses by its greater length (figs. 22-24). In oogonial conceptacles, however, it usually disintegrates before it divides, or after it has divided into two or three cells, and gives rise to considerable mucilaginous substance (figs. 12, 14-16). By this difference in the fate of the tongue cell it is possible to distinguish the antheridial from the oogonial conceptacles in the early stages of development. Hermaphroditic conceptacles resemble the antheridial in that the tongue cell commonly develops into a paraphysis.

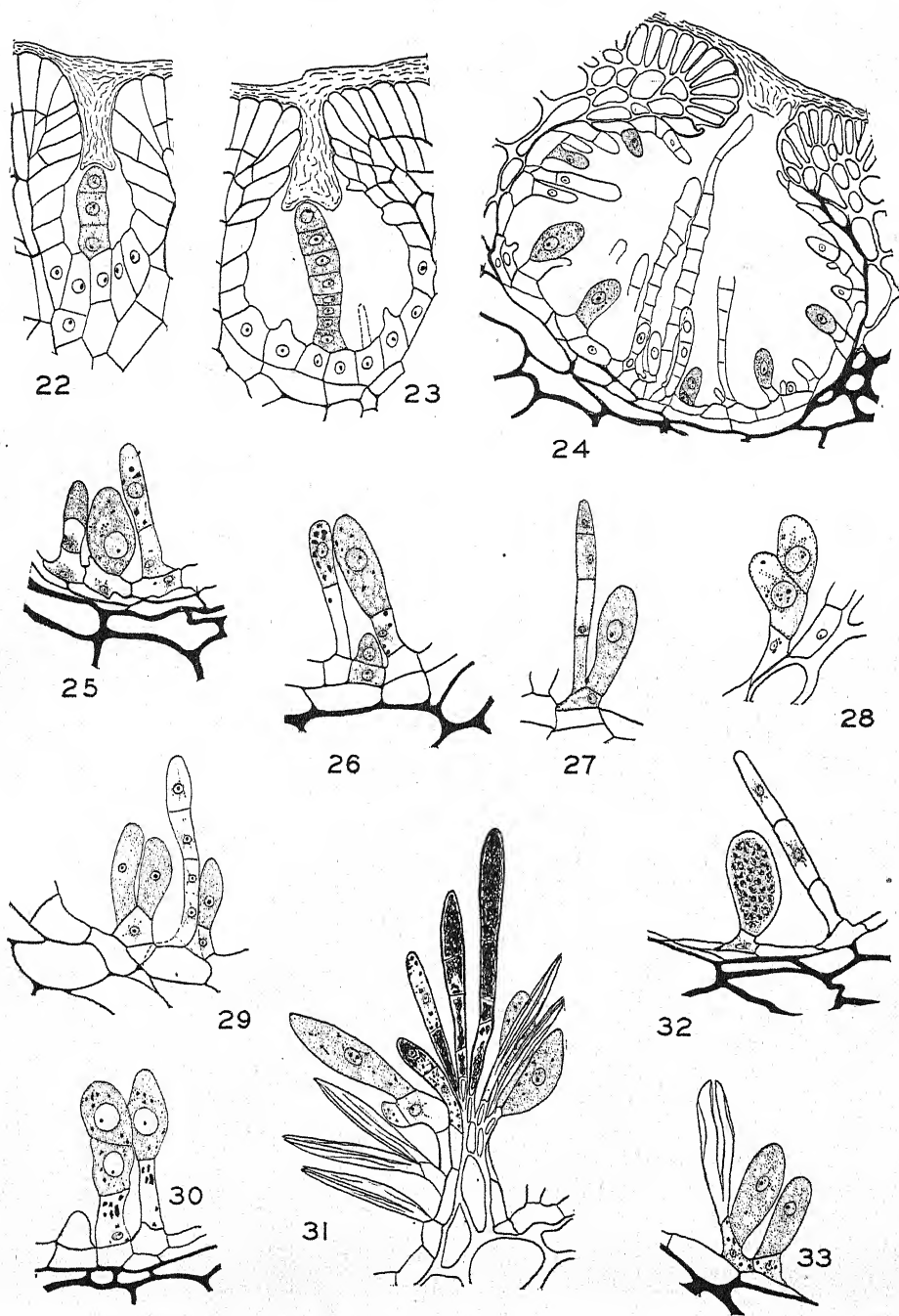
The inner of the two cells formed in the first division of the conceptacle initial has been variously called: the "inner cell" (23), the "basal cell" (18), the "conceptacle initial" (6), and the "true conceptacle initial" (3). To avoid confusion it seems best to designate the original cell as the conceptacle initial and the inner of the two cells resulting from its first division as the inner or basal cell. In *T. turbinata*, as in *Sargassum* and all the other genera so far investigated, division of the basal cell originates the development of the cells lining the conceptacle. This development in *T. turbinata* agrees with that of *Sargassum* as shown by the work of SIMONS and NIENBURG (19), as well as with that of most other genera in which it has been studied. The first division is by a median longitudinal wall (figs. 7, 10), which is followed in the resulting cells by similar divisions in the two planes until about sixteen cells are formed (figs. 8, 9, 11-13). A cross-section at this stage shows that division is of a quadrate type, with no indication that any one cell acts as an initial (fig. 13). This stage is usually followed by a transverse division of the peripheral cells, which are now slightly

raised above the inner, owing to the concavity of the floor of the young conceptacle (figs. 9, 12). By repeated longitudinal division of the inner cells and by one in the peripheral, followed by some transverse divisions, the lining of the conceptacle is finally formed (figs. 14-17).

PARAPHYSES

In all conceptacles the central area of the floor is sterile, producing a group of paraphyses (fig. 24). This feature was first observed by SAUVAGEAU (22) in *Cystoseira* and has since been noted in *Carpophyllum* (7, 3). DAWSON (4) suggested that this feature may be related to the proliferation of branches which originate from within the conceptacles in *Notheia anomala* Bail. and Harv. (9, 30) and in an anomalous form of *Fucus ceranoides* L. (24). Some of these hairs extend to the ostiole or beyond. Paraphyses also originate in the fertile region of the conceptacle, between the antheridia and oogonia, but these are shorter than the basal hairs and decrease in length upward.

The origin of paraphyses in *T. turbinata* is similar to that of other members of the Sargassaceae. A cell in the lining of the conceptacle divides periclinally. The outer of the resulting cells develops a papilla in its free wall (fig. 20). The nucleus then divides and one of the daughter nuclei migrates into the papilla, which becomes separated from its mother cell by a wall commonly oblique to the outer surface (figs. 18, 19). This papilla then enlarges and by trichothallic growth develops into a paraphysis. Occasionally two papillae develop next to each other in adjacent cells, and therefore may appear to have originated by forking (fig. 18). No truly forked hairs have been observed in *T. turbinata* such as were noted by DAWSON (3) in *Carpophyllum*



FIGS. 22-33.—Fig. 22, young antheridial conceptacle showing three-celled tongue cell paraphysis. Fig. 23, antheridial conceptacle showing eight-celled tongue cell paraphysis. Fig. 24, nearly mature antheridial conceptacle showing group of paraphyses in center of floor of conceptacle. Figs. 25-30, young antheridia. Fig. 31, proliferation with tuft of antheridial filaments. Fig. 32, mature antheridium. Fig. 33, young antheridium developing below older empty one.

flexuosum. Branching of paraphyses does not occur except in the short hairs ("periphyses") below the rim of the ostiole (fig. 21). These hairs may be related to antheridial filaments.

ANTHERIDIA

The development of antheridia, or "spermatocysts," among the Sargassaceae has been studied in the following genera: *Sargassum* (23, 16), *Coccophora* (27), *Bifurcaria* (21), and *Cystoseira* (4). The accounts of KUNEDA and TAHARA are the most comprehensive, as they extended their studies to include the cytological features.

The morphological features in *T. turbinata* conform in general with those of the other Sargassaceae; no attempt has been made to study its cytology. The origin of antheridial filaments is the same as that of paraphyses. In fact, at first the two are indistinguishable. Antheridial cells are first distinguishable from sterile cells by their denser cytoplasm and larger nuclei (fig. 25). In their position they show considerable variation. They may be essentially sessile (fig. 25) as in *Sargassum filipendula* (23) and *Cystoseira foeniculacea* (L.) Grev. (4), or stalked (fig. 30) as in *Sargassum horneri* (Turn.) C. Ag. (16), or branches of filaments (fig. 27). Occasionally, antheridia are formed in short rows (figs. 28, 30) as in *Coccophora langsdorffii* (Turn.) Grev. (27). In stalked forms the filament is usually simple at first, but the stalk may later give rise to an antheridium by branching (figs. 29, 31, 33). Thus younger antheridia often appear below the older ones (fig. 33). Rarely, proliferating outgrowths appear that bear a tuft of antheridial filaments (fig. 31). Commonly, a conceptacle simultaneously has antheridia in different stages of development. No proliferation of antheridia up

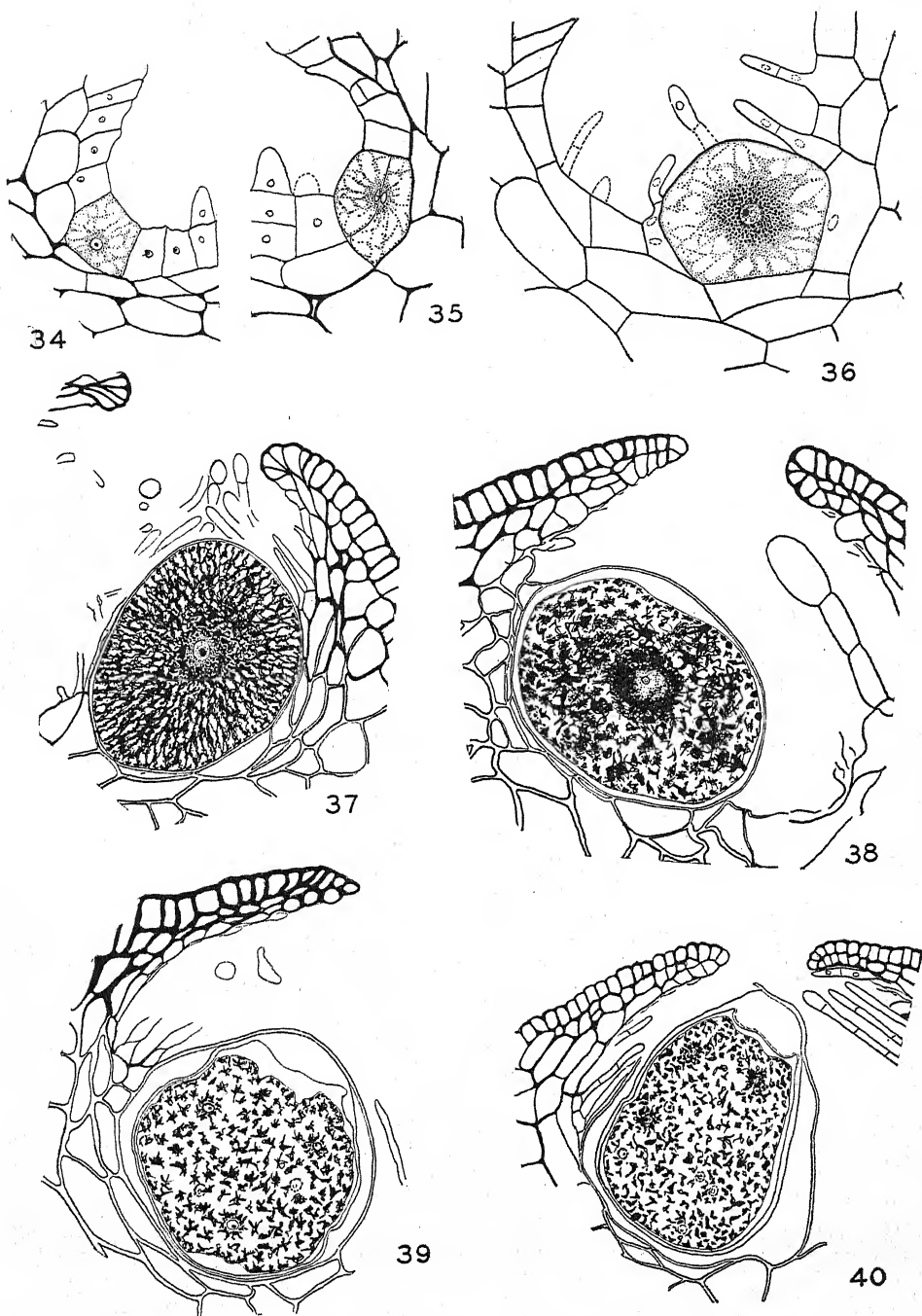
through the older empty ones has been observed, as was noted by DAWSON (4) in *Cystoseira foeniculacea*. In hermaphroditic conceptacles the antheridia appear above the oogonia (protrandrous), as has been reported for other forms.

The sperms are discharged en masse, and apparently this occurs periodically, coinciding with the period of liberation of oogonia, as was observed by ABE (1) in *Sargassum horneri*.

OOGONIA

The cells from which the oogonia or "oocysts" are destined to be formed are located on the lower side of the conceptacle and are derived from the peripheral cells of the lining. They are first distinguishable from the surrounding cells by their larger size and larger nuclei (fig. 34). This position of the oogonial mother cells in *T. turbinata* corresponds with that of other genera investigated. Their arrangement in relation to one another, however, does not agree with that of *Carpophyllum* as reported by DELF (7) and by DAWSON (3). In this genus the arrangement is reported to be spiral, and oogonia are therefore of different ages within one conceptacle. The explanation of this arrangement rests upon the assumption that "the 'conceptacle initial' behaves as a specialized apical initial" (3). In *T. turbinata* there is no indication that the oogonia are spirally arranged or differ markedly in age within one conceptacle—as in *Sargassum* and *Cystophyllum* (25, 26). Furthermore, a transverse section of the early products of the basal cell shows no indication that any initial cell is maintained (figs. 10, 13).

The oogonial initial cell divides periclinally into two cells, the outer of which becomes the oogonium mother cell without further division (fig. 35). The inner



FIGS. 34-40.—Fig. 34, oogonial initial cell. Fig. 35, division of oogonial initial forming oogonial mother cell and stalk cell. Fig. 36, young oogonium. Fig. 37, oogonium just before formation of mesochiton. Fig. 38, oogonium with developing mesochiton. Fig. 39, eight-nucleate oogonium with mesochitinous pad. Fig. 40, eight-nucleate oogonium inside ruptured exochiton.

or "stalk" cell does not function as a stalk in raising the oogonium above the surrounding cells but is soon crushed by the rapidly enlarging oogonium (fig. 37).

During the early development of the oogonium there is no indication that hairs develop on its outer wall, as DAWSON (4) observed in *Cystoseira foeniculacea*. However, as the oogonium expands into the surrounding cells, the paraphyses located near the oogonium come to lie on its outer surface (figs. 36, 37).

The first indication of differentiation of the oogonium from the oogonium mother cell is the appearance of a number of small chromatophores around its nucleus (fig. 36). As the oogonium enlarges these plastids increase in number, size, and extent, eventually becoming irregular in shape and somewhat radiating from the center (fig. 37). The enlarging oogonia exert considerable pressure on the surrounding cells, which become distorted into plates and columns between the oogonia. Owing to the elliptical shape of most of the conceptacles (brought about by elongation of the receptacles), the oogonia are more crowded transversely than longitudinally. Oogonia which have reached their final stage of development before liberation vary somewhat in shape but are commonly elliptical. Their average size is $140 \times 174\mu$. Each oogonium possesses a large nucleus with granular chromatin, which is characteristic of the Sargassaceae. Three to six oogonia are borne in each conceptacle, but five is the most common number.

NUCLEAR MATURATION AND DISCHARGE

The first indication that an oogonium has approached the stage of liberation is the production of a thick mucilaginous layer between the original cell wall and the protoplast (fig. 38). This layer has been called "mesochiton," while the orig-

inal cell wall has been termed the "exochiton." No "endochiton" has been observed in *T. turbinata*, as has been reported in *Cystophyllum flexuosum* (3) and *Cystoseira foeniculacea* (4). In the outer region of the oogonium, the mesochiton thickens more rapidly than elsewhere and forms a distinct cushion or pad (figs. 38, 39). This pad was first observed by KUNEIDA (16) in *Sargassum horneri*. It is no doubt largely responsible for the rupture of the exochiton on one side at the upper end, forming a lid which is hinged on the opposite side (fig. 40). In *T. turbinata* this mesochitonous pad does not seem to slide down the side of the oogonium, as has been observed in *Sargassum horneri* (16), *Carpophyllum flexuosum* (3), and *Cystoseira foeniculacea* (4). The subsequent events leading to the discharge of the oogonium have not been observed, but from the final orientation of the oogonium and the basal attachment of the mucilaginous stalk which holds it they may be visualized as follows. In emerging from the exochiton, owing to the attachment of the mesochitonous pad to the lid of the exochiton, the oogonium is inverted, so that the basal end becomes the distal end in the discharged oogonium (fig. 41). Accompanying this emergence, the mesochitonous pad becomes attenuated into a strand which remains attached to the lid of the exochiton within the conceptacle, thus anchoring the discharged oogonium (fig. 41).

The discharge of oogonia is an interesting feature which was first observed by TAHARA (25) in *Sargassum enerve* C. Ag. He found that the discharge was periodic and simultaneous in all plants and occurred at fortnightly intervals that coincided with the spring tides. The oogonia are discharged in zones, commencing at the base of the

receptacle and progressing upward for each succeeding period. This periodicity was investigated further by INOH (11) in twelve species of *Sargassum*, and he found that all discharge oogonia periodically in relation to the spring tides, although they may fruit at different seasons.¹ Discharge usually takes place 1-3 days after the highest tide. TAHARA (26) found that, in artificial cultures, discharge of oogonia was considerably delayed. He also concluded that in *Sargassum thunbergii* O'Kuntze, discharge of oogonia probably occurs at neap tide, which—if true—represents an exception to all other species in which periodicity has been noted.

Although apparently no special studies have been made on periodicity of oogonial discharge in other genera, it has been noted in *Cystophyllum* (26, 12-14) and in *Turbinaria fusiformis* (28), and indications are that it is also present in *Carpophyllum* (3) and *Bifurcaria* (6). While this phenomenon is no doubt widespread among the genera of the Sargassaceae, and evidently also in some of the Fucaeeae, it must not be concluded that it is universal. TAHARA (27) found in *Cocophora langsdorffii* that, while discharge was simultaneous in all conceptacles, it was not periodic. In *Cystoseira foeniculacea*, DAWSON (4) found evidence of periodicity inconclusive, although indications are that it is present in this species also.

While no special effort was made to determine periodicity in *T. turbinata*, from the fact that the oogonia are discharged in definite successive zones (fig. 2) it may be concluded that the discharge is periodic in this alga also. From

¹ Observations on *Sargassum filipendula*, the common and widespread species of the Atlantic coast of North America, indicate that this species also discharges oogonia at fortnightly periods at the time of spring tides.

examination of receptacles of different ages, it appears that oogonia are discharged in three zones (fig. 2), as has been observed in other forms. Generally, all oogonia are discharged from each conceptacle at one period. Occasionally, however, one may remain behind, owing to some interference in the process or some lack of maturity. That the latter is probably the more common reason is indicated by the fact that this irregularity

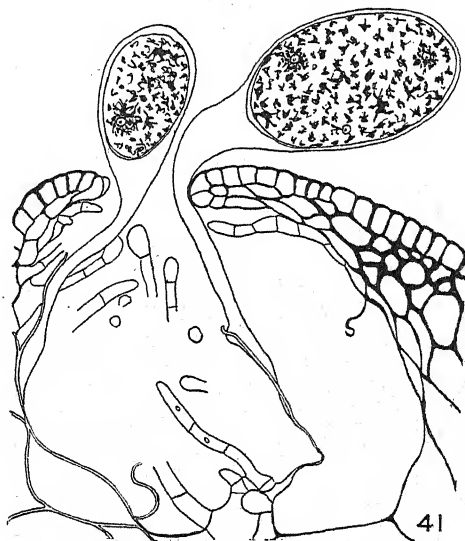


FIG. 41.—Oogonial conceptacle with discharged, attached eight-nucleate oogonia.

is most frequent in the upper (younger) margin of the discharged zone.

The cause or causes of this periodicity in the discharge of oogonia in the Sargassaceae are not definitely known. It has been suggested (6), however, that light and tide variation may be important factors.

Another interesting feature associated with the discharge of oogonia is their attachment. DODEL-PORT (5) first observed that oogonia adhere on the outside of the receptacle in *Cystoseira barbata*. In this species they are simply held

by mucilage to cryptostomatal hairs. A similar situation was found by INOH (14) in *Cystophyllum crassipes* (Mert.) J. Ag. and by OKABE (20) in *C. sisymbrioides* (Turn.) J. Ag. In the majority of instances, however, they are held by a mucilaginous stalk which originates from the mesochiton. While oogonia thus attached had been observed by SIMONS (23) and TAHARA (25), the first one to have actually noted the stalks was NIENBURG (18). The species in which this structure has been observed since that time have been tabulated by DAWSON (4).

Oogonia are included within their individual mesochitonous sheaths during the period of attachment and are in addition collectively imbedded in a copious, transparent, mucilaginous substance (fig. 2). The origin of this substance has not been determined in *T. turbinata*, but it probably comes largely from the paraphyses, an opinion held also by other investigators.

As already stated, NIENBURG (19) was the first to conclude that the nucleus of the oogonium undergoes meiosis, although he did not actually observe the process. Apparently, owing to the rapidity with which it occurs, it is difficult to obtain cytological evidence. TAHARA (29) seems to have been the first and only one who has succeeded in observing meiosis in the oogonium in the Sargassaceae—in his work on the genus *Coccophora*. From circumstantial evidence, however, it is generally assumed that meiosis does take place, and this applies also to the present work on *Turbinaria*. To obtain mitotic or meiotic figures in the Phaeophyceae necessitates special effort in collecting and fixation, which it is hoped can be undertaken at some future time in *Turbinaria*.

In *T. turbinata*, as in the majority of

the members of the Sargassaceae which have been studied, reduction division is followed by another division, resulting in eight nuclei. Exceptions are *Coccophora langsdorfi* (28) and *Bifurcaria laevigata* (Kütz.) Delf and Mitch. (17), which evidently have only four.

As to the location of the oogonium at the time of meiosis—that is, whether it takes place before or after discharge—there seems to be considerable variation in the different genera. This may be more apparent than real, however, because of the difficulty of observing oogonia immediately prior to or during the process of discharge. There is also the possibility that it takes place both before and after discharge in the same plant.

In *T. turbinata* reduction division seems to occur prior to discharge, as a few conceptacles were found which showed oogonia *in situ* with eight nuclei (figs. 39, 40). Of course, the possibility exists that these oogonia were for some reason delayed in being discharged, and therefore represent an abnormality. However, eight-nucleate oogonia within the conceptacle were observed by KUNEIDA (16) in *Sargassum horneri*; and from reports by other observers on other genera it may be concluded that in the majority of forms meiosis takes place in the oogonium prior to discharge. A definite exception is presented by *Coccophora*, however, in which TAHARA (28) observed only a single nucleus in the oogonium at time of discharge.

It has not been possible to determine definitely the method of elimination of the seven supernumerary nuclei from the egg, but—from the stages available—indications are that they disintegrate within the cytoplasm, as occurs in *Sargassum* and in the other species of *Turbinaria* which have been studied.

EMBRYOGENY

The first observations on embryological development in the Sargassaceae were made by SIMONS on *Sargassum filipendula*. She noted that the fertilized egg remains attached to the outside of the receptacle until a several-celled structure has been formed, and that "The first division of the egg in *Sargassum* does not differentiate a rhizoidal region, as in *Fucus* and *Ascophyllum*." She also observed that several rhizoids develop at one end after the embryo has grown into many cells. NIENBURG (18) found in *Sargassum linifolium* that the first wall of the fertilized egg is laid down across the middle and that a later division cuts off at one end a lenticular rhizoidal cell. Subsequent work on the embryogeny of the Sargassaceae has been done mainly by Japanese investigators, who have paid special attention to early segmentation and rhizoidal formation.

In summarizing studies on the embryogeny of the Sargassaceae, the following facts seem to have been pretty well established:

1. In all forms investigated, the developing embryo usually remains attached to the outside of the receptacle until it has developed into a several-celled structure. This change requires a period of 2-3 days.
2. The fertilized egg is first divided into approximately two equal cells across the shorter diameter if the egg is ellipsoidal.
3. At the second division, a small lenticular cell is cut off at one end, although *Sargassum linifolium* may be an exception, for—according to NIENBURG (19)—this occurs at the third division.
4. The embryo proper develops by a

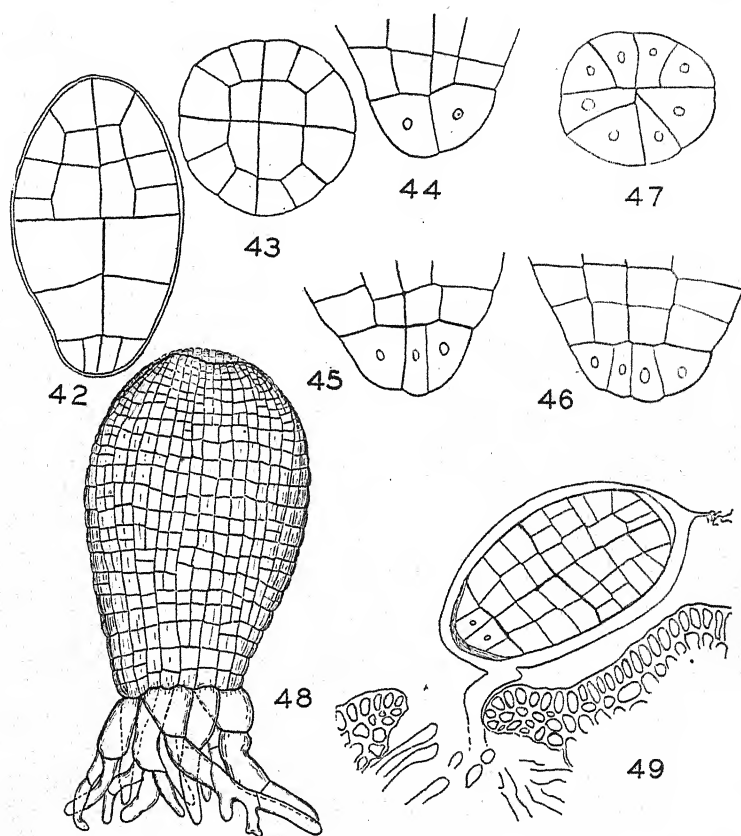
regular quadrate method of cell division until it has become many-celled and an apical cell is established.

5. There is considerable variation among the different genera—and even among the species of one genus (*Sargassum*, 11)—in the number of rhizoids and their formation.
6. Primary rhizoidal cells are formed by longitudinal divisions of the rhizoidal cell. In a few forms, however, a "two-storied" development may result from transverse divisions of the first cells formed (*Cystophyllum*, 26, 20; *Sargassum patens*, 11).
7. The number of primary rhizoids is usually four, eight, sixteen, or thirty-two, with some individual variation.
8. Secondary rhizoids may arise from the basal cells of the embryo proper or from the inner primary rhizoidal cells.
9. The number of primary rhizoids and their method of formation are apparently of no great systematic significance.

In the material of *T. turbinata* used in this study a few embryological stages have been obtained, but unfortunately these do not include the early ones. However, from the stages available (figs. 42, 43) it is apparent that development conforms in general with that of other genera and other species of *Turbinaria* (28, 13). The lenticular rhizoidal cell is divided radially by two perpendicular walls into four approximately equal cells (fig. 44), each of which is divided once by oblique walls (figs. 42, 45, 46), resulting in eight primary rhizoidal cells from which grow eight primary rhizoids. This agrees with the development and number of rhizoids in several species of *Sargassum* (28, 11), and in *Coccophora* (27), *Turbinaria fusiforme* (28), *T. ornata* and

T. filiformis (14). A few advanced embryos were encountered with well-developed primary rhizoids (fig. 48). These are unique in two respects: first, they show forking which has been seen only in *Cystophyllum crassipes* (14); second, they indicate branching, which has not been reported previously. No stages

have been reached. From examination of sections of a number of embryos of *T. turbinata*, it appears that the rhizoidal end is commonly directed toward the ostiole (fig. 49). This would indicate that the rhizoidal end is the original outer end of the oogonium which becomes inverted in the process of emergence.



FIGS. 42-49.—Fig. 42, longisection of young embryo. Fig. 43, transection of young embryo. Figs. 44-46, longisection of rhizoidal cell showing divisions. Fig. 47, transection of rhizoidal cell showing eight primary rhizoidal cells. Fig. 48, many-celled embryo with primary rhizoids. Fig. 49, longisection of embryo showing orientation.

were observed sufficiently advanced to show secondary rhizoids.

In the study of embryogeny in this family there has been some speculation concerning the orientation of the embryo, but apparently no definite conclusions

In conclusion, it might be said that, in its reproductive structures and their development, *T. turbinata* appears to conform closely with that of the majority of genera and species of the family Sargassaceae so far investigated.

Summary

1. *Turbinaria turbinata* occurs in Puerto Rico, mostly on the northern or Atlantic side, on rocky substrata in shallow depressions or to a depth of 5 feet.
2. Plants are commonly of one sex, but not infrequently hermaphroditic as well as antheridial and oogonial conceptacles appear in the same receptacle.
3. Receptacles originate from a triangular apical cell which in longitudinal sections appears lenticular.
4. In origin and development the conceptacles are like those of *Sargassum* and most of the other genera and species of the Sargassaceae which have been studied.
5. In the male conceptacle a normal paraphysis develops from the tongue cell, but in the oogonial conceptacles it disintegrates early and gives rise to a mucilaginous substance.
6. The central area of the floor of the conceptacle is sterile and bears a tuft of unbranched paraphyses, as has been observed in a few other genera.
7. The antheridia vary in position from being essentially sessile to being stalked or as branches of filaments. Occasionally they occur in short rows, as in *Coccophora*.
8. Oogonia develop in the lower re-

gion of the sides of the conceptacle and are approximately of the same age within each conceptacle. Five is the most common number.

9. Eight-nucleate oogonia have been seen *in situ*, indicating that meiosis occurs just before or during emergence.

10. The oogonium develops a mesochiton which in the outer region forms a pad. This pad ruptures the exochiton and elongates into a stalk which anchors the discharged oogonium.

11. The embryo has eight primary rhizoids which are commonly situated at the end proximal to the ostiole.

12. Except for a few minor differences, *T. turbinata* conforms in its reproductive structures to that of *Sargassum* and the majority of other Sargassaceae.

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MEIOSIS IN AUTOTETRAPLOID *LOLIUM PERENNE* IN RELATION TO CHROMOSOMAL BEHAVIOR IN AUTOPOLYPLOIDS¹

W. M. MYERS²

Introduction

The irregularities of meiosis and, consequently, the low fertility of autopolyploids have been attributed by DARLINGTON (7) to the multivalent association of chromosomes during synap-

sis. This hypothesis has been supported by several investigators, particularly KOSTOFF (14, 15). It is evident from the literature that there has been a general tendency to accept multivalent frequency as a criterion of the relative meiotic irregularity of various autopolyploids. KOSTOFF (15) concluded that, in species with short chromosomes, the autopolyploids would tend to be more regular in meiosis because of lower chiasma frequency and, hence, fewer multivalents. This hypothesis was adopted by EARN-

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SHAW (9) to account for the fertility of tetraploid sea plantains allied to *Plantago maritima* L. From studies of meiosis in tetraploids and in hybrid triploids, he concluded that the 4x plants were autotetraploids. The quadrivalent frequency was low, never more than two per cell, a fact that he attributed to the low chiasma frequency.

In *Dactylis glomerata* L., which behaves cytogenetically like an autotetraploid ($x = 7$), MYERS (30) and MYERS and HILL (35) found, however, that variation in quadrivalent frequency was not a reliable measure of the irregularity of meiosis. Unequal disjunction of the quadrivalents at anaphase I occurred only infrequently in this species. On the other hand, unpaired chromosomes at metaphase I were found in considerable frequency in most plants. Because of their tendency to lag and divide equationally at anaphase I and to be left in the cytoplasm at telophase I and II, these unpaired chromosomes were an important contributing factor to the formation of aneuploid gametes. The frequency of unpaired chromosomes was not significantly correlated with quadrivalent frequency among unrelated plants of *Dactylis* (34, 30). Quadrivalent frequency was positively correlated with chiasma frequency, which in turn was negatively correlated with incidence of univalents at metaphase I. It was concluded, therefore, that reduction in quadrivalent frequency resulting from reduced numbers of chiasmata would be accompanied by an increase in univalents at metaphase I (30). Hence, plants with low chiasma frequency would be on the average less regular in meiosis than those with high frequency.

Data from induced autotetraploids of *Lolium perenne* L., reported in this paper, provide further information re-

garding irregularities of meiosis in autopolyploids. These data are of special interest for comparison with meiotic behavior in naturally occurring autotetraploid grasses, such as *Dactylis glomerata*.

MATERIAL AND METHODS.—The material consisted of pairs of diploid and tetraploid clones obtained by HILL and MYERS (12) from chimera plants produced by treatment of germinating seeds of *Lolium perenne* with solutions of colchicine (25). In this report, the related diploid and tetraploid clones are identified by the number of the original seedling, followed by 2x and 4x, respectively, as—for example—C50-2x and C50-4x. Microsporocyte material was collected from twelve pairs of diploid and tetraploid clones growing in adjacent rows in the field. Later, material was collected in the greenhouse from five pairs of clones, including three of those grown in the field and two additional ones.

The microsporocyte material was killed and fixed in acetic alcohol and stored in the fixing solution in a household refrigerator. All data were collected and photomicrographs were made from fresh aceto-carmin smear slides.

Experimental results

TIMING BALANCE IN MEIOSIS

In contrast to most diploid plants of *Lolium perenne* and to normal plants of other grass species that have been examined by the writer, well-spread diakinesis preparations were never obtained from the autotetraploid plants of *L. perenne*. Contraction of the chromosomes, attributed to despiralization by SWANSON (44), was delayed relative to the progression of the stages of meiosis. The chromosomes were still long and

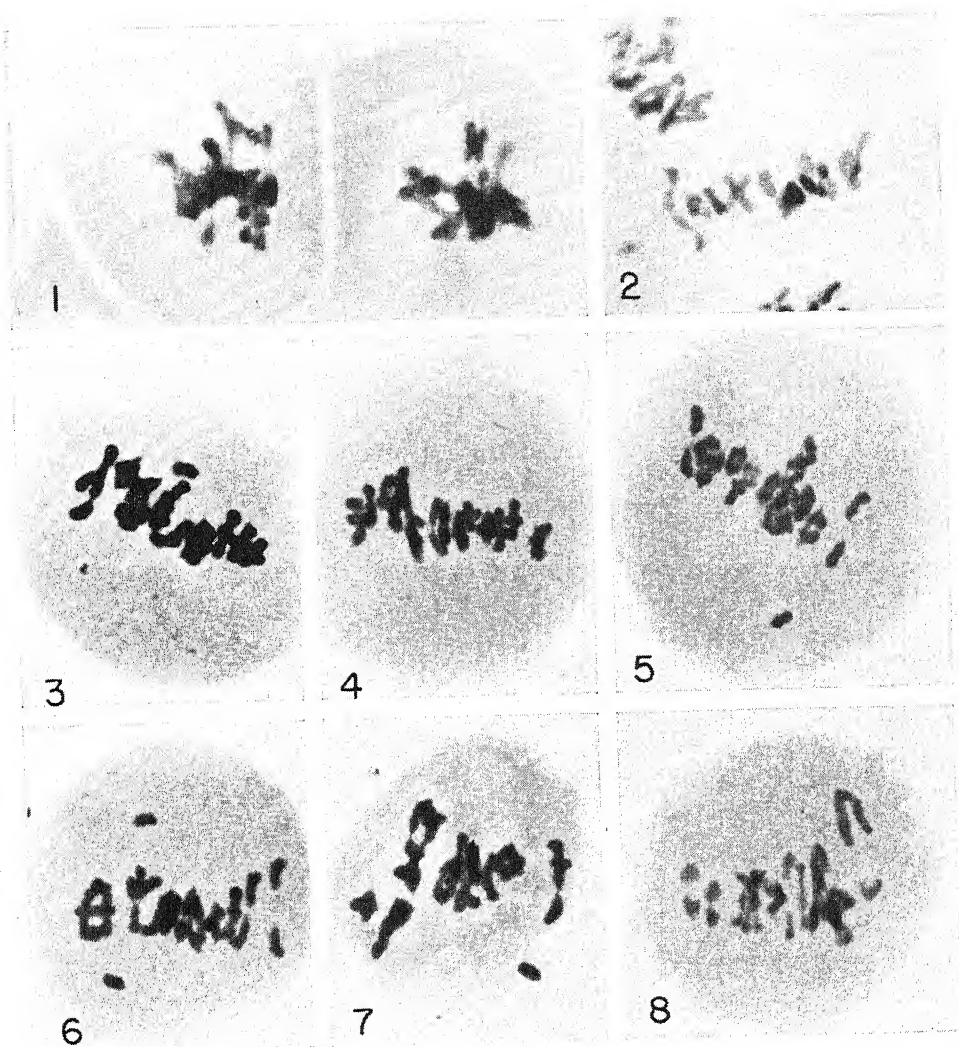
tangled when congression on the equatorial plate commenced. In most plants, contraction of the chromosomes continued and apparently was completed at metaphase I, prior to the initiation of anaphase I. In these plants, well-spread and readily interpreted metaphase I sporocytes were obtained (figs. 2-8). In one clone (C108-4x), however, contraction was not completed and the chromosomes did not become oriented in an orderly manner at metaphase I (fig. 1). In this clone, interpretation of metaphase I was extremely difficult. This difference in synchronization of chromosome contraction, congression on the equatorial plate, and separation at anaphase I was not observed in C108-2x compared with other diploid clones. Behavior similar to that in C108-4x was observed in C190-4x. In this clone orientation was so disorderly and the chromosomes so tangled that interpretation of pairing was not possible in any sporocyte at metaphase I.

The clone C108-4x differed from other clones also in the progression of meiotic stages among florets of the same spikelet and among spikelets of the same inflorescence. In *L. perenne* the lowermost spikelet is the youngest and the stage of development of the microsporocytes is progressively more advanced from spikelet to spikelet as the top of the spike is approached. Within the spikelet the basal floret is the most advanced in development and the terminal floret the least. In each pair, except C108, the rate of progression (development of the microsporocytes) between adjacent florets and adjacent spikelets in the tetraploid was indistinguishable from that in the diploid. In C108, progression was noticeably slower in the tetraploid than in the diploid. This appears to provide further evidence

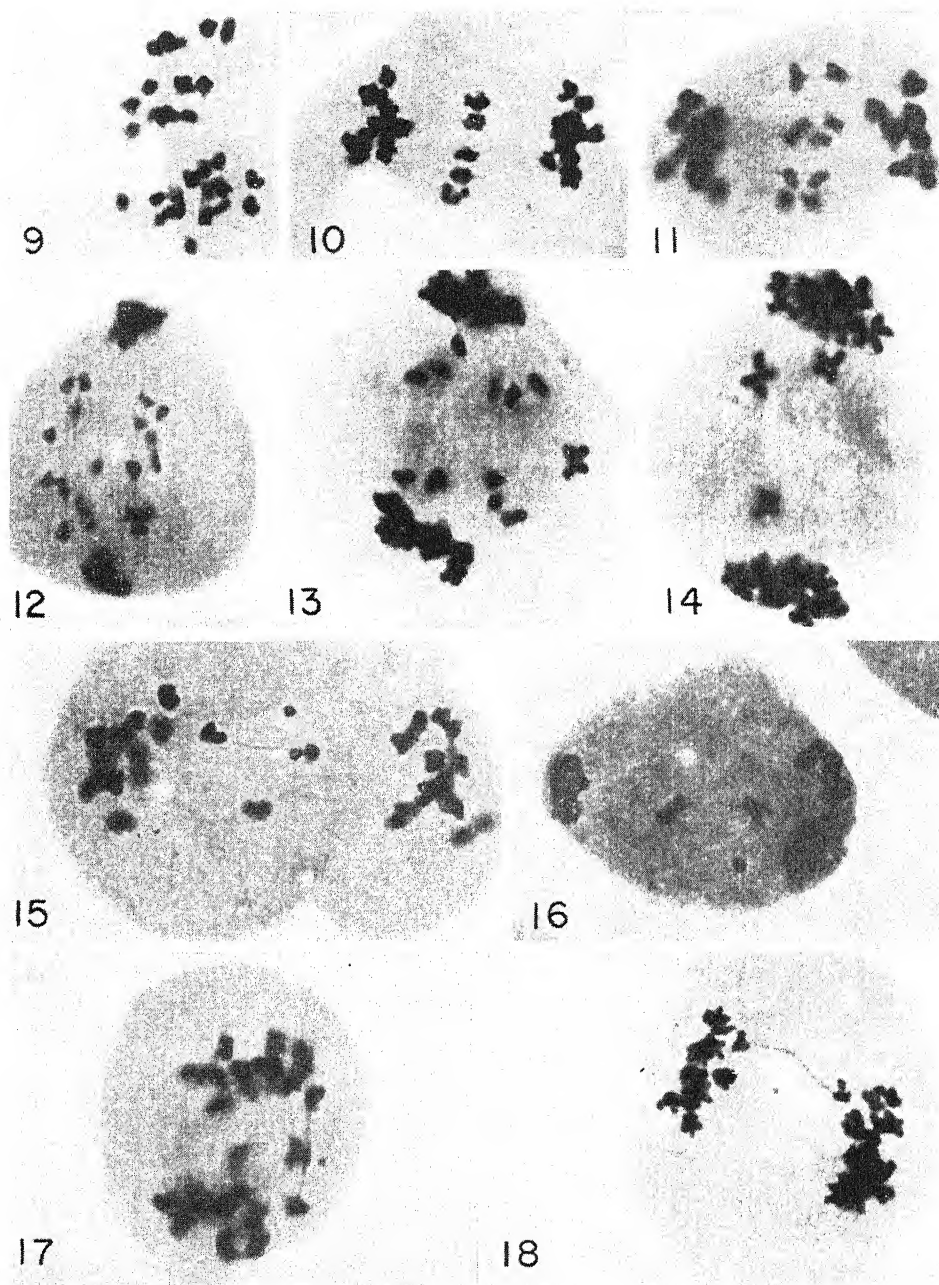
of the upset of the orderly process of meiosis resulting from chromosomal reduplication in this clone.

CHIASMA FREQUENCIES OF DIPLOID AND TETRAPLOID CLONES

UPCOTT (46) concluded that the chiasma frequency per chromosome might be lower in polyploids than in comparable diploids, owing to delay of pairing resulting from the larger nucleus of the polyploid. Evidence was obtained from autotetraploid *Primula kewensis* that supported this hypothesis. Furthermore, UPCOTT (46) cited nine reports from the literature in which the chiasma frequency of the polyploid was lower than that of the comparable diploid. Similar results were obtained by RICHARDSON (41) in *Setcreasea brevifolia*, ANDERSON and SAX (1) in *Tradescantia*, KOSTOFF (16) in *Petunia hybrida*, and PETO (37) in induced autotetraploid *Hordeum vulgare*. Contrary results were obtained by LEVAN (19) in the *Allium paniculatum* group. EARNSHAW (9), in studies of sea plantains allied to *Plantago maritima*, obtained data on chiasma frequencies from five diploid and four tetraploid plants; on the average there was little difference between them. In *Dactylis*, MÜNTZING (23) reported that the chiasma frequency per chromosome in the diploid *D. aschersoniana* was about as high or only slightly lower than in the tetraploid *D. glomerata*. The validity of most of these comparisons is impaired by one or both of two factors: (a) data were collected from too few sporocytes, and (b) the polyploids were not genetically comparable with the diploids. In five of the nine cases cited by UPCOTT (46), chiasma frequency of the polyploid was determined from one or two cells. The inadequacy of such data is evident when the variation in chiasma frequency



FIGS. 1-8.—Fig. 1, metaphase I with irregular orientation. Figs. 2, 3, 4, types of quadrivalents at metaphase I. Fig. 5, trivalent and univalent at metaphase I. Fig. 6, types of quadrivalents at metaphase I; three chromosomes of one quadrivalent oriented toward one pole. Fig. 7, univalent and trivalent at metaphase I. Fig. 8, disjunction of quadrivalent with three chromosomes moving to one pole.



FIGS. 9-18.—Fig. 9, anaphase I showing 13-15 distribution of chromosomes. Fig. 10, five lagging univalents at anaphase I showing equational split. Fig. 11, five univalents dividing at anaphase I. Fig. 12, daughter chromosomes from lagging univalents at telophase I. Fig. 13, daughter chromosomes from lagging univalents and one laggard with centromere not divided. Fig. 14, undivided laggards at interphase I. Fig. 15, short dicentric bridge and large acentric fragment in tetraploid clone. Fig. 16, dicentric bridge and acentric fragment in diploid clone from same pair as tetraploid clone of fig. 15. Fig. 17, dicentric bridge and acentric fragment of intermediate size. Fig. 18, long dicentric bridge and small acentric fragment.

among sporocytes of the same anther is considered. The importance of heritable differences in chiasma frequency among plants of the same species has been emphasized by MYERS (30) and MYERS and HILL (35). The necessity for controlling genetic differences was recognized also by KOSTOFF (16), whose data were obtained from diploid and tetraploid branches of the same plant, and by PETO (37), whose data were obtained from diploid and tetraploid florets of the same spike.

The clones used in these investigations provide material for a critical study of the effects of chromosomal reduplication on chiasma frequency, since the tetraploid clones should differ cytogenetically from their related diploid clones only in number of alleles of each gene and amount of chromatin material in the nucleus. For determination of chiasma frequencies, an average of twenty complete metaphase I sporocytes was analyzed for each tetraploid clone and an average of twenty-six sporocytes for each diploid clone. It was evident from these data (table 1) that the effect of chromosomal reduplication on chiasma frequency was not constant for all clones. The chiasma frequency of the field-grown tetraploid clones varied from 76 to 103% of the frequency of the respective diploid clones. In the absence of replication, the significance of these differences cannot be determined directly. In studies of variations in chiasma frequencies using replicated clones of *Dactylis glomerata*, MYERS (30) found that $2 \times \text{S.E. of the difference for a single determination} = 0.08$. Using this value as a guide in comparing the results obtained in the present investigation, it is evident that in the field material the diploid and tetraploid clones of five pairs did not differ in chiasma frequency. In each of six pairs the tetraploid clone

had a lower chiasma frequency than the diploid, and the differences probably were significant. The greatest reduction was in C108, which—as indicated earlier—showed evidence of an upset in timing balance in meiosis as a result of chromosome doubling.

Data from the greenhouse material were generally in agreement with those obtained from the field material. The differences were small and probably not significant in four pairs, while in the fifth pair the tetraploid may have been significantly lower than the diploid in chiasma frequency. Three of the pairs in which no difference was obtained were included in the field material, where the differences likewise were not significant. The correlation coefficient of chiasma frequency of the tetraploid clones with that of the diploids was 0.316 (14 D/F), a nonsignificant value.

In contrast to *D. glomerata*, in which almost all the chiasmata are completely terminalized, both terminal and subterminal chiasmata occur in diploid *L. perenne* (26). Subterminal chiasmata were found also in the induced autotetraploids (figs. 3-7). Quantitative data on degree of terminalization were not collected in these investigations, but the tetraploid clones did not differ noticeably from the diploids in this regard.

QUADRIVALENTS AT METAPHASE I

In average quadrivalent frequency per sporocyte, the tetraploid clones varied from 2.92 to 4.83, with an average for all clones (including two determinations from each of three of the clones) of 3.96 (table 1). The range in number of quadrivalents is similar to values obtained for unrelated plants of *D. glomerata* by MYERS and HILL (34, 35) and MYERS (30).

DARLINGTON (6, 7) has diagrammed the ten types of quadrivalents possible in an autotetraploid. Six of these types (DARLINGTON'S numbers 11, 12, 13, 15, 16, and 17) were found in this material. Among the 1252 quadrivalents recorded,

times (0.9%); type 13 (figs. 2, 6) was observed sixteen times (1.3%); type 15 was found seventeen times; and type 16 occurred sixty-four times (5.1%). A preponderance of symmetrical types is expected in a species with a chiasma fre-

TABLE 1

COMPARATIVE DATA ON MEIOTIC BEHAVIOR IN PAIRS OF DIPLOID AND TETRAPLOID CLONES OF *LOLIUM PERENNE*; EACH PAIR DERIVED BY VEGETATIVE PROPAGATION FROM A SINGLE COLCHICINE-TREATED GERMINATING SEED

CLONE NO.	X-TA PER CHROMOSOME			IV PER SPORO- CYTE IN 4X	MI WITH UNIVALENTS (%)		AI WITH LAGGARDS (%)		QUARTETS WITH MICRONUCLEI (%)	
	2X	4X	Ratio		2X	4X	2X	4X	2X	4X
	Field-grown material									
101.....	1.80	1.75	97	4.06	1.7	30.6	2.2	44.6	2.1	56.6
102.....	1.66	1.71	103	3.26	0.9	17.3	0	31.4	2.0	41.8
108.....	1.96	1.49	76	3.67	1.3	51.8	0	55.6	0	54.0
116.....	1.89	1.83	97	4.31	1.9	28.8	0.9	26.6	2.8	25.0
166.....	1.82	1.65	91	4.05	1.3	38.1	1.4	59.3	0.5	62.8
190.....					37.9	45.5	54.6	95.2	58.5	95.8
198.....	1.77	1.76	99	4.44	1.2	22.1	1.7	45.4	6.4	54.5
214.....	1.63	1.36	83	2.92	1.8	26.2	0	17.4	1.2	53.0
218.....	1.74	1.74	100	3.57	1.8	24.8	1.9	17.3	1.4	40.3
241.....	1.87	1.74	93	4.83	1.3	23.9	2.3	45.0	0.8	27.1
250.....	2.05	1.81	88	3.30	0	19.1	0	26.4	0.5	14.6
251.....	1.94	1.80	93	3.71	1.3	19.2	1.0	25.0	2.7	49.1
Av.....	1.83	1.69	93	3.83	4.4	29.0	5.5	40.8	6.6	47.9
	Greenhouse-grown material									
50.....	1.84	1.69	92	4.50	4.8	27.1	0.8	43.4	0.5	34.4
101.....	1.67	1.74	104	4.07	4.1	35.9	3.9	48.4	5.4	45.2
183.....	1.69	1.79	106	3.68	4.0	20.6	0.2	21.3	0.7	28.0
198.....	1.92	1.85	96	4.62	4.3	26.2	1.1	51.1	3.1	55.4
218.....	1.84	1.90	103	4.44	0.9	18.3	2.3	20.3	1.1	17.1
Av.....	1.79	1.79	100	4.26	3.6	25.6	1.7	36.9	2.2	3.6
Grand av.....	1.82	1.73	95	3.96	4.4	28.0	4.4	39.6	5.6	44.4

the most frequent types were those with symmetrical arrangements, that is: the simple ring, type 17 (61.8%); and simple chain, type 11 (29.4%). These types are shown in figures 2, 4, 5, 6, and 7. Type 12 (fig. 3) was observed twelve

quency such as found in *L. perenne*, since only one chiasma per chromosome arm is required for their formation, whereas two chiasmata per chromosome arm (in at least two chromosomes) are required for formation of the other types

of quadrivalents. Quadrivalent frequency was significantly correlated with chiasma frequency in this material ($r = 0.517$, $P < 0.05$).

The unequal disjunction at anaphase I of the chromosomes of multivalents has been reported by DARLINGTON (7) to be the principal cause of instability and low fertility in autopolyploids. In this material unequal disjunction apparently occurred frequently. At metaphase I quadrivalents were found in which the members were oriented in a manner to suggest the probability that three chromosomes would pass to one pole and one chromosome to the other at anaphase I. In figure 6 the second quadrivalent from the left is oriented in this manner. At early anaphase I, quadrivalents were observed in which the separation of three chromosomes to one pole was evident (fig. 8, quadrivalent on extreme right). This unequal disjunction of members of quadrivalents would be expected to result in the production of aneuploid daughter nuclei at telophase I, and hence of aneuploid gametes. At anaphase I, the number of chromosomes in both groups could be determined with certainty in forty sporocytes in which no lagging occurred. There was a 14-14 distribution in twenty-one (52.5%), 13-15 in sixteen (40%), and 12-16 in three (7.5%). A sporocyte with a 13-15 distribution is shown in figure 9.

OCURRENCE AND BEHAVIOR OF UNIVALENTS AT METAPHASE I

The incidence of univalents was determined from an average of 249 metaphase I sporocytes in the diploid clones (table 1). The frequency was low in all clones except C190-2x, being similar to that reported previously for diploid plants of this species (26). In C190-2x, 37.9% of the metaphase I sporocytes

had two (or occasionally four) unpaired chromosomes.

The frequency of sporocytes with univalents in the tetraploid clones, determined from an average of 224 metaphase I sporocytes, was considerably higher than in the diploids, varying from 17.3 to 51.8%. In some instances there was a single univalent plus a trivalent (figs. 5, 7), but more commonly two or more univalents were found (fig. 6)—the incidence of trivalents being relatively low in this material.

The frequency of univalents in C190-4x (45.5%) was lower than expected from the behavior of C190-2x. Since metaphase I was extremely difficult to analyze in this clone, it is probable that some univalents were overlooked and that this value is too low. This assumption is supported by the high incidence of lagging at anaphase I (95.2% of the sporocytes with laggards).

Variations among tetraploid clones in incidence of asynapsis at metaphase I may be attributed in part to variations in chiasma frequency. The coefficient of correlation between these characters was -0.516 ($P < 0.05$). On the other hand, metaphase I univalent frequency was not correlated with number of quadrivalents ($r = 0.048$). Also, metaphase I univalent frequency of the tetraploid clones was not significantly correlated with metaphase I univalents of the diploids ($r = 0.255$, $P < 0.10$) when data from C190 were omitted in the calculation.

Lagging univalents, most of which were dividing equationally (figs. 10, 11), were found at anaphase I in some sporocytes in each of the tetraploid clones, the frequency varying from 17.3 to 95.2% for the different clones. The daughter half-chromosomes from the lagging and dividing univalents began to separate at

late anaphase I (fig. 11) and were observed to be moving toward the poles at the end of anaphase I or early telophase I (figs. 12, 13). The infrequency of micronuclei in the cytoplasm during interphase compared with laggards at anaphase I indicated that a majority of the daughter half-chromosomes were included in the interphase nuclei. Rarely, a lagging univalent was observed in which the centromere apparently had not divided (fig. 13). These univalents were left in the cytoplasm at interphase (fig. 14).

Lagging univalents have been reported in several species to have resulted from the failure of metaphase I univalents to move to the poles at anaphase I. In this material frequency of anaphase I laggards was significantly correlated with metaphase I univalents ($r = 0.67$, $P < 0.01$). This value of r , although significant, is lower than that reported by MYERS (30) and MYERS and HILL (35) for plants of *D. glomerata*. Furthermore, in most tetraploid clones there was a higher frequency of sporocytes with laggards at anaphase I than with unpaired chromosomes at metaphase I (table 1). DARLINGTON (5, 6) and MYERS (32) have reported that laggards may result from incomplete disjunction of multivalents. If so, there might be expected a positive correlation between quadrivalent frequency and anaphase I laggards. In the tetraploid clones, r was 0.486 compared with r of 0.497 necessary for P of 0.05.

The daughter half-chromosomes from anaphase I laggards were observed to lag at anaphase II, and many were left in the cytoplasm to form chromatin clumps or—sometimes—micronuclei. The frequency of quartets with chromatin clumps and micronuclei was significantly correlated with frequency of

laggards at anaphase I ($r = 0.548$, $0.05 > P > 0.02$).

EFFECTS OF INVERSIONS ON RANDOMNESS OF CHROMOSOMAL ASSOCIATION DURING PROPHASE

It has been proposed by MÜNTZING (22), DARLINGTON (7), and others that some species that behave cytologically as allopolyploids may have arisen from autopolyploids by a process of chromosomal differentiation. According to DARLINGTON (7), gene rearrangements rather than intragenic changes would be the principal factor in such differentiation. SKIRM (42) reported an autotetraploid *Tradescantia* with predominantly bivalent pairing that he attributed to structural heterozygosity and to doubling following fertilization. There arises, therefore, the problem of the relative importance of inversions in inhibiting the randomness of prophase pairing in autopolyploids. Dicentric chromatid bridges and acentric fragments that apparently had resulted from crossing-over in inversions were found at anaphase I in most plants of *D. glomerata* by MYERS and HILL (34). Such configurations were reported also by GILES (11) in autotetraploid *Tradescantia*. These observations indicate either that the inversion was present in the simplex or triplex condition, or that—if it was duplex—it did not limit synapsis to inverted and normal pairs in the chromosome section in which it occurred.

Bridges and fragments were observed at anaphase I in each of the diploid clones used in these investigations, the frequency varying from 0.74 to 11.12% of the sporocytes (table 2). Such configurations also were observed in each of the tetraploid clones. Since the inversions were duplex in the tetraploids, the results indicate that inverted segments

paired at prophase with homologous normal chromosomes. This seems unlikely, however, since the length of the segments of normal chromosomes. There is the possibility that the colchicine dicentric chromatid and the size of frag-

TABLE 2

FREQUENCIES OF ANAPHASE I SPOROCTES WITH DICENTRIC CHROMATID BRIDGE AND ACENTRIC FRAGMENT IN DIPLOID AND TETRAPLOID CLONES OF *LOLIUM PERENNE* AND THE FREQUENCIES EXPECTED IN THE TETRAPLOIDS, ASSUMING RANDOM PAIRING OF INVERTED AND NORMAL CHROMOSOMES WITH AND WITHOUT CORRECTION FOR DIFFERENCES IN CHIASMA FREQUENCY

CLONE NO.	X-TA OF 4X IN PERCENTAGE OF 2X	PERCENTAGE SPOROCYTES WITH BRIDGE PLUS FRAGMENT			
		2X observed	4X		
			Observed	Calculated	
				Un- corrected	Corrected for X-ta
Field-grown material					
101.....	97.2	0.74	4.05	0.98	0.95
102.....	103.0	1.34	3.36	1.78	1.83
108.....	76.0	3.18	0.56	4.23	3.21
116.....	96.8	6.60	4.84	8.78	8.50
166.....	90.6	11.12	4.44	14.79	13.40
198.....	99.4	5.68	5.15	7.55	7.50
214.....	83.4	4.22	2.04	5.61	4.68
218.....	100.0	3.12	7.82	4.15	4.15
241.....	93.0	1.55	1.25	2.06	1.92
250.....	88.3	0.20	5.75	0.27	0.24
251.....	92.8	5.05	4.54	6.72	6.24
Av.....		3.89	3.98	5.17	4.78
Greenhouse-grown material					
50.....	91.8	2.07	10.86	2.75	2.52
101.....	104.2	3.39	6.26	4.51	4.70
183.....	105.9	2.26	3.18	3.00	3.18
198.....	96.4	4.66	12.86	6.20	5.98
218.....	103.3	4.05	11.04	5.39	5.57
Av.....		3.29	8.82	4.37	4.39
Grand av.....		3.70	5.49	4.92	4.66

treatment caused inversions in the tetraploid, which, being in the simplex condition, would be expected to pair with the homologous segment of one of the

ment were similar in different sporocytes of the same clone and in the diploid and tetraploid clones of the same pair (figs. 15, 16), whereas considerable differences

were found among clones from different pairs (figs. 17, 18).

If normal and inverted segments pair at random during prophase in the autotetraploid, the frequency of sporocytes with bridges and fragments should be 1.33 times the frequency in the diploid, provided that chiasma formation occurs with equal ease. The observed frequencies of bridges and fragments, and those calculated with and without correction for differences in chiasma frequency at metaphase I in the tetraploid clones, are shown in table 2. In nine of the tetraploid clones the frequency of bridges and fragments was greater than calculated, while in seven it was less. The great variation in behavior observed in the different pairs may have resulted, in part at least, from the relatively small numbers of anaphase I sporocytes observed—an average of 148 per tetraploid clone and 223 per diploid clone. More data are required for a critical evaluation of the effects of inversions, but it is evident that inversions of the size found in this material did not appreciably inhibit randomness of chromosome pairing.

Discussion

The data indicate that there are three major types of meiotic irregularity in autotetraploid *Lolium perenne*, namely, (a) the members of the quadrivalents frequently disjoin unequally, resulting in 13-15 and 12-16—instead of the normal 14-14—distributions at anaphase I; (b) there are cases of incomplete disjunction of the members of quadrivalents resulting in lagging and dividing univalents at anaphase I; and (c) there is a high frequency of univalents at metaphase I that tend to lag and divide equationally at anaphase I and to be left in the cytoplasm at telophase I or telophase II. Each of these kinds of irregularity has

been found in other autopolyploid species. Unequal disjunction from quadrivalents has been reported commonly (6, 7, 3, 17, 18, 20, 45, 46, 39, 43, and others), while relatively little attention has been given to the importance of unpaired chromosomes at metaphase I. This latter factor has been emphasized particularly by MYERS and HILL (35) and MYERS (30), who found it to be the most important kind of irregularity in *Dactylis glomerata*. The importance of improper disjunction from quadrivalents as a source of anaphase I laggards was indicated by COOPER's (4) results with induced autopolyploid *Medicago sativa*. The results of the present investigations are in agreement with MYERS' (30) conclusions that the feature of meiotic irregularity of greatest importance varies with the species and that multivalent frequency alone will not always be a reliable criterion of stability and fertility of an autotetraploid.

Experimentally produced autopolyploids have, in general, been low in fertility. As stated previously, DARLINGTON (7), KOSTOFF (14), and many others have attributed this reduced fertility primarily to the formation during meiosis of multivalents that disjoin unequally at anaphase I. On the other hand, MÜNTZING (22) and RANDOLPH (40) have proposed that the infertility of autopolyploids results primarily from physiological disturbances and upsets in genic balance accompanying chromosomal reduplication. SPARROW, RUTTLE, and NEBEL (43), working with *Antirrhinum*, found differences in fertility among autotetraploids of different origin that were not associated with differences in quadrivalent frequency, and FISCHER (10) reported similar results from autotetraploids of maize. Likewise, MYERS and HILL (35) found that quadrivalent fre-

quency was not correlated with differences in fertility among plants of inbred progenies of *D. glomerata*. On the other hand, SPARROW *et al.* (43) reported that sterility was a concomitant of, but probably was not conditioned by, a significant difference in 18-14 distributions at anaphase I. MYERS and HILL (35) found that differences in fertility were significantly correlated with frequency of unpaired chromosomes at metaphase I, laggards at anaphase I, and micronuclei in the quartets in *Dactylis*. These features of meiotic irregularity accounted for approximately 16% of the variations in fertility, whereas MYERS (29) reported that about 38 and 23%, respectively, of the variability of seed set under bag and with open-pollination in that experiment could be attributed to heritable factors. Thus, it appears that meiotic irregularities and genetic differences both play a role in conditioning variations in fertility in *Dactylis*. It is significant, however, that variation in quadrivalent frequency did not have a measurable influence on fertility, yet multivalent frequency has been accepted commonly as a criterion of meiotic irregularity of autopolyploids. The inadequacy of such a measure is emphasized further by comparison of meiotic behavior of *D. glomerata* with that of autotetraploid *L. perenne*.

The infertility of experimental autopolyploids has led to the assumption (7, and others) that autopolyploids, as such, would seldom persist in nature as sexually reproducing species. DAWSON (8) concluded that autopolyploid species have persisted in nature by developing a mechanism giving regular disjunction at meiosis, or by developing some asexual type of reproduction.

In the perennial forage grasses, several species and chromosome races within

species behave cytogenetically as autopolyploids. These include *D. glomerata* (21, 38, 33, 27), the tetraploid race of *Agropyron cristatum* (33), *Arrhenatherum elatius* (13, 33), *Anthoxanthum odoratum* (13), *Hordeum bulbosum* (2), and *Phleum pratense* (36, 24, 28, 31). These species are sufficiently fertile to permit their distribution commercially by seed. Of these, *D. glomerata* has been studied most extensively from the standpoint of meiotic behavior. Comparisons of meiosis in *Dactylis* and in autotetraploid *L. perenne* should provide evidence regarding some of the factors that have enabled *D. glomerata* to persist in nature as a sexually reproducing autotetraploid.

The range in quadrivalent and chiasma frequency among plants of *D. glomerata* is similar to the range among clones of autotetraploid *L. perenne*. On the other hand, unequal disjunction of the chromosomes of quadrivalents occurred commonly in *L. perenne* but was relatively infrequent in *D. glomerata*. Furthermore, improper disjunction of quadrivalents resulted in laggards at anaphase I in *L. perenne*, whereas MYERS and HILL (34, 35) found that quadrivalent frequency was not significantly correlated with frequency of anaphase I laggards in *D. glomerata*. And MYERS (30), using the analysis of covariance, showed that all significant differences in frequency of laggards could be attributed to variations in incidence of metaphase I univalents.

The differences in disjunction of quadrivalents in the two species may be attributed, in part at least, to differences in types of quadrivalents and in terminalization of the chiasmata. In *Dactylis* the quadrivalents were almost invariably simple rings and chains, while more complicated types were found in *Lolium*. The quadrivalents with symmetrical

arrangements might be expected to disjoin more regularly in a 2-2 manner at anaphase I. The complete terminalization of chiasmata in *Dactylis* probably also is a factor contributing to the greater regularity of disjunction of the quadrivalents, for—as DARLINGTON (7) has pointed out—quadrivalents with terminal chiasmata have a greater flexibility, permitting a more orderly orientation on the equatorial plate. Also, the simplest and quickest separation at anaphase I should be among the chromosomes with terminal chiasmata (7).

A further difference in meiotic regularity between *Dactylis* and *Lolium* autotetraploids is in the greater incidence of unpaired chromosomes at metaphase I in the latter. This difference cannot be attributed to lower chiasma frequency, since the two species were similar in that regard. It seems probable that the disturbance in timing relationships resulting from chromosome doubling in *Lolium* may have been an important factor in the higher univalent frequency.

One of the important features of the results obtained in these investigations was the lack of relationship between behavior of the diploid and of the related tetraploid clone. This lack was evident in the upset of timing in Cro8-4x compared with the other tetraploids, and in the insignificance of correlation coefficients between diploid and tetraploid clones in chiasma frequency, asynapsis at metaphase I, laggards at anaphase I, and micronuclei in the quartets.

Summary

1. Meiotic behavior was studied in pairs of diploid and tetraploid clones of

Lolium perenne L. obtained by vegetative propagation from chimera plants resulting from treatment of germinating seeds with solutions of colchicine.

2. As compared with the diploid clones, there was in the tetraploids a delay of chromosome contraction relative to the onset of metaphase I and in two clones absence of orderly orientation on the equatorial plate prior to initiation of anaphase I.

3. In some pairs the chiasma frequency of the tetraploid clone was lower than in the diploid, while in other pairs the differences were not significant.

4. There were three major types of meiotic irregularity in the tetraploid clones: (a) the members of the quadrivalents frequently disjoined unequally at anaphase I; (b) lagging and dividing univalents at anaphase I resulted in some cases from incomplete disjunction of quadrivalents; and (c) there was a high incidence of univalents at metaphase I that tended to lag and divide equationally at anaphase I and to be left in the cytoplasm at telophases I and II.

5. Variations in meiotic behavior among the tetraploid clones were not significantly correlated with variations among the diploid clones.

6. The frequencies of anaphase I sporocytes with dicentric bridges and acentric fragments in the tetraploid clones did not differ greatly from the frequencies calculated—assuming random pairing of inverted and normal chromosomes—from those obtained in the comparable diploid clones.

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EXPERIMENTAL POLYPLOIDY AND RUBBER CONTENT IN TARAXACUM KOK-SAGHYZ¹

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Introduction

Polyploidy is known to affect the storage of chemical products in many plants. Vitamin C content has been reported higher in polyploid than in diploid forms of the following species: apple (1), tomato (9), and rose hips (6). KOSTOFF and AXAMITNAJA (4) found more nitrogen and water in tetraploids than in related diploids of tomato. Pigment content of *Antirrhinum* and *Torenia* blossoms was found by STRAUB (10) to increase with the degree of polyploidy (2n, 3n, 4n, 6n, and 8n). STRAUB also demonstrated that the carotene content per cell in the yellow spot of blossoms of the latter genus increases from diploid through octoploid, the increase in pigment content being greater than the relative increase in cell volume. RANDOLPH and HAND (8) reported that doubling of chromosome number in yellow corn causes a

40% increase in carotenoid content. Triploid and tetraploid forms of hemp have a higher marihuana toxicity per unit weight of dry leaves than related diploids (12, 14). Recently, GUSTAFSON (3) has reported an opposite effect in marigold and the cherry tomato; namely, that tetraploids have less growth hormone than the diploids from which they were derived.

Polyploidy has also been shown by numerous workers to effect an increase in the dimensions of plant structures. In general, tetraploids differ from diploids in having thicker stems, broader leaves, larger flowers, thicker roots, larger pollen grains and seeds, etc. It seemed of value, therefore, to induce chromosome doubling in *Taraxacum kok-saghyz*, the Russian dandelion, and to study the effect of such doubling on rubber yield. The rubber in this species is in the latex of the root, which—as in our domestic species—is a more or less branched tap root. An increase in the percentage of rubber or an increase in the size of the root, or

¹ Co-operative project with Rubber Plant Investigations, U.S. Department of Agriculture, Agricultural Research Administration, Bureau of Plant Industry, Soils, and Agricultural Engineering.

both, would be desirable characteristics from the standpoint of rubber yield.

Polyploidy in the genus *Taraxacum* is also of theoretical interest in relation to apomixis. In this genus, diploid species reproduce in the normal sexual manner; natural polyploid species, on the other hand, are apomictic. On the female side of the polyploids there is a failure of one of the meiotic divisions (diplospory, 2), resulting in the formation of an unreduced egg. This egg undergoes development without fertilization (diploid parthenogenesis). It was thus of interest to observe the effect of autopolyploidy on reproduction in *T. kok-saghyz*, which is a sexual diploid with chromosome numbers of $n = 8$, $2n = 16$.

The Russian workers KOSTOFF and TIBER (5) and NAVASHIN and GERASSIMOVA (7) obtained tetraploid plants of this species by colchicine treatment. They did not report changes in rubber percentage or in root size, and they apparently observed no effect on the reproductive processes in the tetraploid forms. Many workers in this country, including Drs. G. L. STEBBINS, L. F. RANDOLPH, and ERNST ARTSCHWAGER, also have succeeded in obtaining tetraploids in this species by colchicine treatment (personal communications).

MATERIAL AND METHODS.—Seeds of *T. kok-saghyz* were received from the U.S. Department of Agriculture, Rubber Plant Investigations, on May 16, 1942, under plant quarantine no. 143960. These were treated by immersion for 1, 2, and 4 days in 0.05%, 0.1%, 0.2%, 0.4%, and 0.8% aqueous solutions of colchicine in covered Syracuse dishes at room temperature. The seeds were rinsed in water before being planted in seed pans in the greenhouse. Surviving seedlings were transplanted to 3-inch and later to 4-inch pots.

Successfully treated plants were identified by microscopic examination of pollen grains. Pollen determinations were subsequently checked by making chromosome counts from root tips of many second-generation polyploid plants, using the section-smear technique (11).

Roots were prepared for rubber analysis in the following manner: they were dug, carefully washed, immersed in boiling water for 20 seconds, and then decapitated at the level of the crown. Immersion in boiling water was used at the suggestion of Dr. RANDOLPH, to coagulate the latex and thus prevent "bleeding" when the top was removed. The roots were then blotted with paper towels to remove excess water and weighed. They were allowed to dry at room temperature until a constant weight was reached and then were reweighed to determine the relative loss of weight in drying. Rubber analyses were made by Dr. SAM WILDMAN of the Department of Agriculture, using a turbidimetric method.

Results

The results of treatment of seeds of *T. kok-saghyz* are given in table 1. Of the 123 treated plants which flowered, nineteen were found to be tetraploid or predominantly tetraploid, on the basis of pollen size. Pollen of untreated controls and of unsuccessfully treated plants averages about 28μ in diameter, that of induced tetraploids about 36μ (fig. 1). In addition to having larger pollen grains, successfully treated plants usually also are distinguishable by their thicker, broader leaves, thicker flower stalks, and larger buds and flowers.

Table 1 shows a decrease in the number of plants surviving, both with increasing concentration of colchicine and with duration of treatment. Few plants survived concentrations of 0.4% and

0.8%; also, relatively few survived the 4-day treatment. The highest percentages of successfully treated plants (27.3% and 44.4%, respectively) were obtained from treatments in 0.1% and 0.2% colchicine solutions for 2 days.

T. kok-saghyz is highly self-sterile and rarely sets seed in a screened greenhouse (13); this is true for diploids and tetraploids alike. Abundant seed is secured, however, when flower heads from two different plants are rubbed together.

Chromosome counts were made from root tips of many of the plants to verify the pollen determinations. Diploid, triploid, and tetraploid figures are shown in figure 4.

The second-generation tetraploid seedlings show the usual polyploid characters, but to an extreme degree (fig. 3). Their leaves are broader and shorter than the corresponding diploids; they also are thicker and somewhat less deeply incised. The tetraploids produced

TABLE 1

RESULTS OF TREATMENT OF SEEDS OF *T. KOK-SAGHYZ* IN AQUEOUS SOLUTIONS OF COLCHICINE;
75-100 SEEDS TREATED IN EACH INSTANCE. PLANTS WHICH FLOWERED WERE CHECKED
FOR CHROMOSOME DOUBLING BY POLLEN DETERMINATIONS

COLCHICINE CON- CENTRATIONS (%)	DURATION OF TREATMENT											
	One day				Two days				Four days			
	No. plants surviving	Plants flowering			No. plants surviving	Plants flowering			No. plants surviving	Plants flowering		
		2n	4n	% 4n		2n	4n	% 4n		2n	4n	% 4n
0.05.....	58	9	1	10.0	66	30	3	9.1	33	8
0.1.....	70	20	2	9.1	54	16	6	27.3	11	1
0.2.....	42	12	3	20.0	30	5	4	44.4	5
0.4.....	4	5	2
0.8.....	4	1
Total.....	178	42	6	12.5	155	53	13	19.7	49	9

The seed from tetraploid crosses is clearly larger than that from diploid crosses (fig. 2).

Many crosses were made among the original induced tetraploids and between these and untreated diploids. From such crosses, tetraploid, triploid, and diploid seed was obtained. Part of the tetraploid seed was forwarded to R. W. HENDERSON at the U.S.D.A. Rubber Plant Field Laboratory, St. Paul, Minnesota; the remainder, together with triploid and diploid seeds, was sown in seed pans in the greenhouse at Cold Spring Harbor.

larger and more leafy rosettes than the diploids throughout the period of these tests.

For comparison of root weight and rubber content, two different lots of seed were planted: the first was small, consisting of seed from six diploid and three tetraploid crosses; it was started 12/19/42 and was grown until time of harvest (7/10/43) in pots in the greenhouse. Mean rubber percentage and fresh root weights for nineteen diploid and nineteen tetraploid plants from this planting are given in table 2.

The second comparison between diploids and tetraploids was started 4/30/43 and concluded 12/1/43. This included a greater number of plants (thirty-six diploids and forty-three tetraploids), which, after being started in the greenhouse, were transplanted to a small experimental plot outdoors. Each plant was given approximately 1 square foot of space. In addition to root weight and rubber percentage, this group was compared also as to water content of the roots, by weighing roots both fresh and dry. Comparisons of the field-grown diploids and tetraploids are given in table 2.

Mean fresh-root weight is greater for tetraploids than for diploids in both the greenhouse and the field comparisons. Even though the numbers are small, the differences are significant in both cases (diff./s.e. = 2.14 and 3.65, respectively). Among the field plants, diploid roots lost $73.23 \pm 0.49\%$ by weight in drying, and the tetraploids lost $72.45 \pm 0.57\%$ (table 2). These differences are not significant (diff./s.e. = 1.04) and indicate a closely comparable water content for $2n$ and $4n$ roots of this species.

The results of rubber analyses differ under the two sets of conditions. The greenhouse comparisons indicate a lower percentage of rubber in the tetraploids than in the diploids. This difference approaches significance (diff./s.e. = 1.91). The field-grown tetraploids exceed the field-grown diploids both in root weight and in rubber percentage. In this case the difference between diploid and tetraploid in rubber percentage is clearly significant (diff./s.e. = 3.90). When data for both greenhouse and field-grown plants are combined (table 2), the tetraploids are seen to have significantly higher mean root weights (16.77 ± 1.68 gm. to 9.21 ± 0.93 gm.) and a somewhat higher rubber percentage ($2.95 \pm 0.28\%$

to $2.14 \pm 0.26\%$). These differences are probably both significant (diff./s.e. = 3.94 for root weight and 2.13 for rubber percentage). The largest single fresh root observed, 56.5 gm., was a tetraploid; the highest individual rubber percentage, 8.9%, occurred in a diploid.

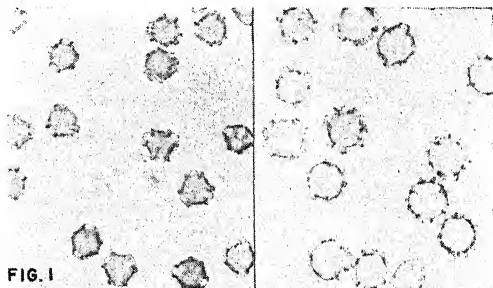


FIG. 1

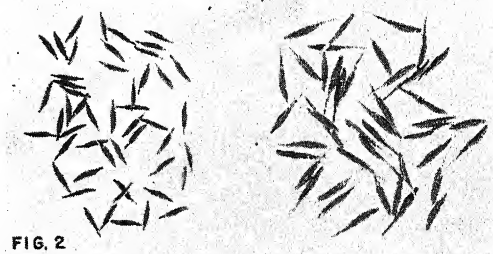


FIG. 2

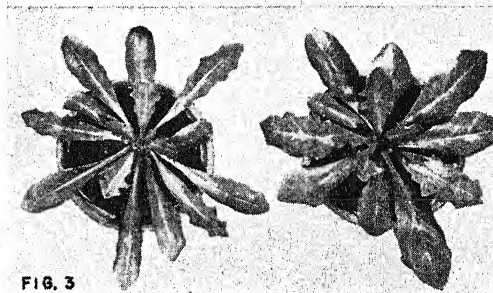


FIG. 3

FIGS. 1-3.—Comparison of diploid and tetraploid plants of *T. kok-saghyz*. Diploid at left and tetraploid at right: Fig. 1, pollen grains. Fig. 2, seeds. Fig. 3, seedlings, age 68 days.

What is of greater importance ultimately than either root weight or rubber percentage, taken separately, is their combination; that is, how much rubber a tetraploid plant yields as compared with a diploid. Rubber yields have been cal-

culated for these plants by multiplying dry-root weights in grams by rubber percentages of individual roots (table 2). The combined diploids produced an average of 0.060 ± 0.009 gm. of rubber per plant; the combined tetraploids produced an average of 0.195 ± 0.035 gm., nearly 3.3 times as much.

To be a high yielder it is obvious that a plant must have both a large root and one containing a high percentage of rubber. The correlations between root weight and rubber percentage are there-

best diploid. The higher correlation coefficient (0.561) between root weight and rubber percentage in the field-grown tetraploids is clearly evident in table 4.

In contrast to the natural polyploids of this genus, the colchicine-induced polyploids show no signs of apomictic reproduction. Reproduction is proved to be sexual by results of the $4n \times 2n$ and reciprocal ($2n \times 4n$) crosses. Triploids, with chromosome numbers of 24, have been obtained from both crosses. In apomictic species the chromosome number

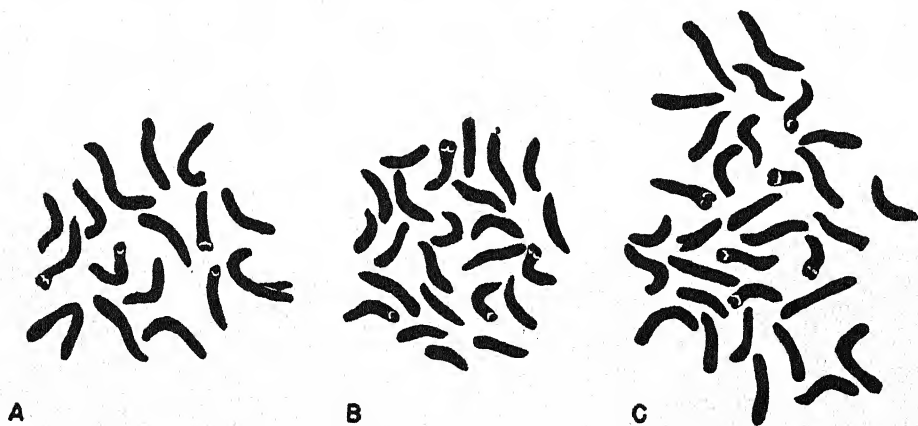


FIG. 4.—Chromosomes from root tips of polyploid plants of *T. kok-saghyz*: A, diploid ($2n = 16$); B, triploid ($3n = 24$); C, tetraploid ($4n = 32$).

fore important. These are given in table 3. There is some positive correlation between these characters in all cases except the greenhouse tetraploids, where a low negative correlation is obtained. Since the numbers are small, however, these values are of questionable significance.

In table 4, dry-root weights, rubber percentages, and yields for the ten best diploids and the ten best tetraploids are recorded. These comparisons show that the most productive tetraploid produced more than four times as much rubber as the most productive diploid (1.131 to 0.263 gm.). The poorest of the ten tetraploids produced more rubber than the

of the offspring is that of the female parent, regardless of the pollen used. Pollen of the experimental tetraploids is well formed (fig. 1) and viable, as indicated by its functioning on diploid females to produce triploid offspring. Pollen of natural polyploids, although most likely a result rather than a cause of apomixis, is invariably aborted to a high degree.

Colchicine-induced polyploids of *T. kok-saghyz* are highly self-sterile, as are diploids, and set seed very rarely when grown in a screened greenhouse. No accurate data are available on the degree of relative self-sterility of diploids and tetraploids of this species, but the in-

TABLE 2
RELATIVE WEIGHTS, RUBBER PERCENTAGES, AND CALCULATED RUBBER YIELDS OF DIPLOID AND TETRAPLOID ROOTS OF T. KOK-SAGHYZ

Material	No. of plants	No. diff. families	Mean fresh-root wt. (gm.)	Range mean fresh-root wt. (gm.)	Mean dry-root wt. (gm.)	Loss of wt. in drying (%)	Mean rubber, dry roots (%)	Range rubber, dry roots (%)	Calculated rubber yield† per plant (gm.)
Greenhouse plants { 2n... { 4n...	19 19	6 3	8.23 ± 0.74 10.71 ± 0.89	4.13-17.62 4.41-21.20	2.20 ± 0.20* 2.95 ± 0.25*	2.13 ± 0.51 1.04 ± 0.26	0.0 - 8.9 0.0 - 4.4	0.033 ± 0.013 .029 ± .007
Diff./s.e.			2.14		2.34	1.91	1.60
Field plants { 2n... { 4n...	36 43	12 13	9.73 ± 1.37 19.45 ± 2.28	0.5 - 31.4 2.35-56.5	2.72 ± 0.40 5.71 ± 0.73	73.23 ± 0.49 72.45 ± 0.57	2.15 ± 0.29 3.79 ± 0.30	0.15-6.9 0.15-7.1	.063 ± .012 .208 ± .047
Diff./s.e.			3.65		3.60	1.04	3.90	4.18
Combined green-house and field plants { 2n... { 4n...	55 62	18 16	9.21 ± 0.93 16.77 ± 1.68	0.5 - 31.4 2.35-56.5	2.54 ± 0.27 4.87 ± 0.54	2.14 ± 0.26 2.95 ± 0.28	0.0 - 8.9 0.0 - 7.1	.060 ± .009 0.195 ± 0.035
Diff./s.e.			3.94		3.88	2.13	3.75

* Calculated from fresh-root weights, assuming same relative water loss as in field plants.

† Rubber percentage X dry-root weight (gm.), individual plants.

frequency of seed-setting in both forms in a screened greenhouse indicates that neither is apomictic. Plants of two naturally occurring polyploid and apomictic species, *T. officinale* and *T. megalorrhizon*, when grown in the same greenhouse with

is more complex and apparently involves a series of evolutionary steps. The fact, however, that—apparently without exception—the many natural polyploids are apomictic points to polyploidy as an essential to the evolution of apomixis in this genus.

TABLE 3

CORRELATION COEFFICIENT OF FRESH ROOT WEIGHT VS. RUBBER PERCENTAGE FOR 2n AND 4n PLANTS OF *T. KOK-SAGHYZ*

Plants	Greenhouse	Field
2n.....	0.327±0.205	0.118±0.164
4n.....	-0.117±0.226	0.561±0.105

Discussion

From the standpoint of rubber yield, these comparisons place the tetraploid form in a very favorable position. Its roots are significantly larger, and its rubber percentage is equal to or slightly superior to that of diploids. The lower percentage of rubber in the greenhouse-

TABLE 4

CALCULATED RUBBER YIELDS FOR TEN MOST PRODUCTIVE DIPLOID AND TEN MOST PRODUCTIVE TETRAPLOID PLANTS OF *T. KOK-SAGHYZ*

RANK	DIPLOID			TETRAPLOID		
	Dry root wt. (gm.)	Rubber (%)	Calculated rubber yield (gm.)	Dry root wt. (gm.)	Rubber (%)	Calculated rubber yield (gm.)
1.....	8.35	3.15	0.263	16.76	6.75	1.131
2.....	6.49	3.75	0.243	15.43	6.30	0.972
3.....	2.51	8.90	0.223	14.30	5.10	0.729
4.....	4.63	3.60	0.167	15.95	4.05	0.646
5.....	2.48	6.30	0.156	9.87	5.55	0.548
6.....	2.30	6.50	0.150	9.41	5.70	0.536
7.....	7.82	1.80	0.141	9.52	5.40	0.514
8.....	1.63	6.90	0.112	9.89	4.05	0.401
9.....	3.11	3.50	0.109	6.50	5.40	0.351
10.....	4.20	2.25	0.095	7.57	4.34	0.329
Mean.....	4.35	4.67	0.166	11.52	5.26	0.616

T. kok-saghyz, produced a full set of seed in every flower head.

The experimental triploids, probably because of irregularities in chromosomal disjunction, are highly cross-sterile as well as self-sterile. These forms essentially are capable of vegetative propagation only. If apomixis were a direct response to decreased sexual fertility of polyploids, it would be expected to appear in these triploid individuals. That it does not indicates that the cause of apomixis

grown tetraploids probably should not be taken too seriously, for the following reasons: (a) The number of plants in the greenhouse tests is small; (b) harvesting was done in July, when rubber storage is known to be low and erratic; and (c) the plants were pot-grown, and therefore grew under unfavorable conditions. The negative correlation between root size and rubber percentage for this particular group of plants may indicate that greenhouse conditions adversely affect

rubber storage in the larger-rooted tetraploids.

The total number of plants studied in these tests is not large—certainly not so large as one would like for a comparison of diploids and tetraploids in a species as heterozygous as *T. kok-saghyz*. However, an effort was made to draw the plants from as wide a base as possible: eighteen families are represented among the fifty-five diploids and sixteen families among the sixty-two tetraploids. The usual method of obtaining comparable 2n and 4n samples from heterozygous stock—that of treating part of the plant with colchicine and then collecting seeds from both the diploid and tetraploid portions—is not applicable in a self-sterile species such as this. If diploid and tetraploid blossoms were obtained on the same plant, these would have to be outcrossed, and the favorable basis of comparison would thus be lost. If rare cases of selfing could be achieved, the problem still would not be solved, for such offspring would almost surely suffer deleterious effects from inbreeding, perhaps differentially.

The problem of relative set of seed in diploid and tetraploid stocks under field conditions has not been studied adequately. This is an important practical consideration, of course, since the fertility of tetraploids is usually lower than that of diploids. Dr. STEBBINS reports a relatively high set of seed but poor fertility from his 4n stocks of *T. kok-saghyz* (personal communication). Our experience with hand-pollinations of greenhouse plants, however, indicates a high degree of fertility among the tetraploid plants, possibly almost as high as among the diploids. The ease with which *T. kok-saghyz* can be propagated vegetatively, however, may make high fertility of less importance in this species than in some others.

The tetraploid plants appeared to suffer less from the phenomenon of “summer dormancy” in these trials than did the diploids. The 4n rosettes were larger and leafier and showed better growth during the summer months than the 2n rosettes. Whether the tetraploids will maintain this advantage in vegetative vigor under more favorable conditions is not known; admittedly the climate and soil of Long Island are not the best for culture of this species.

It is evident that the preceding results should be interpreted as suggesting, rather than as proving, superiority of the tetraploid as a rubber producer in this species. Large-scale field trials, under optimum conditions, would seem to be desirable.

Summary

1. Diploid and tetraploid races of *Taraxacum kok-saghyz* have been obtained by colchicine treatment.

2. Nineteen greenhouse-grown tetraploids had significantly larger roots but a lower rubber percentage than a similar number of greenhouse-grown diploids. Forty-three field-grown tetraploid plants had both significantly larger roots and higher rubber percentage than a group of thirty-six comparable diploids. Combined data from greenhouse and field tests show the tetraploids to have larger roots (16.77 ± 1.68 gm. to 9.21 ± 0.93 gm., fresh weight) and a somewhat higher rubber percentage ($2.95 \pm 0.28\%$ to $2.14 \pm 0.26\%$) than the diploids.

3. The water content of diploid and tetraploid roots did not differ significantly.

4. A low positive correlation between root weight and rubber percentage was observed for field-grown diploids and tetraploids, and for greenhouse-grown diploids. A slight negative correlation was observed for the greenhouse-grown tetraploids.

5. On the basis of calculated rubber yield (dry weight of roots \times percentage of rubber), the diploids produced an average of 0.060 ± 0.009 gm. of rubber; the tetraploids an average of 0.195 ± 0.035 gm., or more than three times as much. The best single tetraploid pro-

duced more than four times as much rubber as the best single diploid.

6. No indication of apomixis was observed among the experimental triploids or tetraploids in these studies.

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NATURE AND RATE OF DEVELOPMENT OF ROOT SYSTEM OF *GONOLOBUS LAEVIS*¹

JOHN C. FRAZIER

Introduction

This is the fifth of a series of reports on growth habits of noxious perennial weeds of central United States being conducted by the Kansas Agricultural Experiment Station. A study (2) of the nature and rate of development of field bindweed established the scope and methods of procedure. The second investigation (3) dealt with hoary cress, the third (4) with

Russian knapweed, and the fourth (5) with dogbane. The present study is concerned with climbing milkweed, *Gonolobus laevis* Michx., a rather important weed pest locally abundant in certain cultivated fields of the eastern two-thirds of the state of Kansas. Its nature as a weed in Kansas has not changed in recent years, HITCHCOCK and CLOTHIER (8) having described it in 1898 as "not widespread in Kansas but locally very abundant. Quite a pest in corn fields in

¹ Contribution no. 464, Department of Botany, Kansas Agricultural Experiment Station.

certain localities." According to GATES (6) the weed has not spread westward appreciably in the state in the last 40 years. GATES (personal communication, 1944) believes it is becoming more abundant within its area of distribution, particularly in central Kansas.

Environmental conditions and methods

SOIL DATA.—All plants² considered in this paper were taken from soil, the profile of which is essentially the same as that described by FRAZIER (2), except that it has two narrow layers of compacted material which may have influenced water penetration. These layers, each approximately 1 inch thick, were at the 26- and 32-inch depths. Both profiles were described by Dr. J. C. HIDE of the Kansas Agricultural Experiment Station.

METEOROLOGICAL DATA.—The monthly and annual temperature and precipitation data for the first 10 months of 1944 compared with the long-time average of these factors are given in table 1. The summer of 1944 had maximum temperatures of 100° F. or higher on 4 days, as compared with 38 such days in 1937, when the comparable study was made on field bindweed; 15 in 1941, when the comparable study was made on hoary cress; 5 in 1942, when the comparable study was made on Russian knapweed; and 9 in 1943, when the comparable study was made on dogbane. The average annual number of days having temperatures of 100° F. or higher during the 50 years, 1889–1938, is 15. The average monthly precipitation for the first 10 months of 1944 was 4.40 inches, as compared with the monthly average of 2.86 inches for these months during the

83-year period, 1858–1940. The total annual rainfall for 1944 was 49.57 inches, only 1.25 inches below the maximum record of 50.82 inches in 1915. Some of the precipitation for the first 10 months of 1944 fell as heavy rains. There were two 24-hour periods in April with 1.94 and 1.96 inches, one in May with 1.79 inches, one in July with 1.82 inches, two in August with 1.84 and 5.86 inches, and

TABLE 1
MONTHLY AND ANNUAL TEMPERATURE (IN °F.)
AND PRECIPITATION (IN INCHES) AT
MANHATTAN, KANSAS, 1944

MONTH	TEMPERATURE		PRECIPITATION	
	Mean*	83-year average 1856–1940†	Total*	83-year average 1858–1940†
Jan.....	35.9	27.0	1.00	0.75
Feb.....	35.2	31.5	.85	1.14
Mar.....	38.1	42.5	4.52	1.48
Apr.....	49.9	54.5	8.92	2.67
May.....	68.4	64.6	3.69	4.31
June.....	75.5	74.5	2.92	4.50
July.....	77.3	79.5	6.81	4.25
Aug.....	79.0	77.5	11.65	3.89
Sept.....	68.7	69.3	2.53	3.44
Oct.....	58.5	56.5	1.15	2.16
Annual mean or total...	55.2	54.2	49.57	30.97

* Meteorological data obtained from U.S. Weather Bureau, Kansas Section.

† Computed by Dr. A. B. CARDWELL of Kansas State College.

one in September with 2.03 inches—making a total of seven such periods, each with a rainfall of more than 1.75 inches.

METHODS.—The plants were grown on a small plot of land free from other noxious weeds and from sodium chlorate. The soil is a fairly typical Geary silt loam representative of the wind-deposited soil of the upland of this locality. The plot had been cultivated a decade earlier but had not been disturbed recently.

Seeds of climbing milkweed were

² The term plant as used in this paper refers to all the growth from a single seed. The term shoot is applied to the individual leafy stems commonly called plants in control studies.

planted on May 5, 1944. Fifteen seeds were placed $\frac{1}{4}$ inch below the surface at each of seventeen points which were so spaced that six of the plants were 14 feet distant from any other and the remainder were separated by 8 feet. May 19, 1944, was taken as the date of emergence for the series, since one seedling emerged at each of fourteen planting points on that date; all seedlings emerging before and after were removed. The area was kept free of all other vegetation, so the only competition for water and plant nutrients, if any existed, was among the plants of the milkweed.

The root systems were excavated at intervals ending November 10, 1944, by a modification of the trenching method developed by WEAVER (10) as described by FRAZIER (2). Efforts were made to keep the root system as one organic entity; if a root broke, the two ends were immediately tied together. Measurements of the plant parts were made and recorded, so that as illustrated they occupy the same relationship, one part to another, as they did in the soil, except that in figures 1, 2, 3, 4, and 6 all lateral roots are arranged in one plane. While as many as possible of the finer roots were obtained, it is not contended that a large portion of them was secured.

Observations

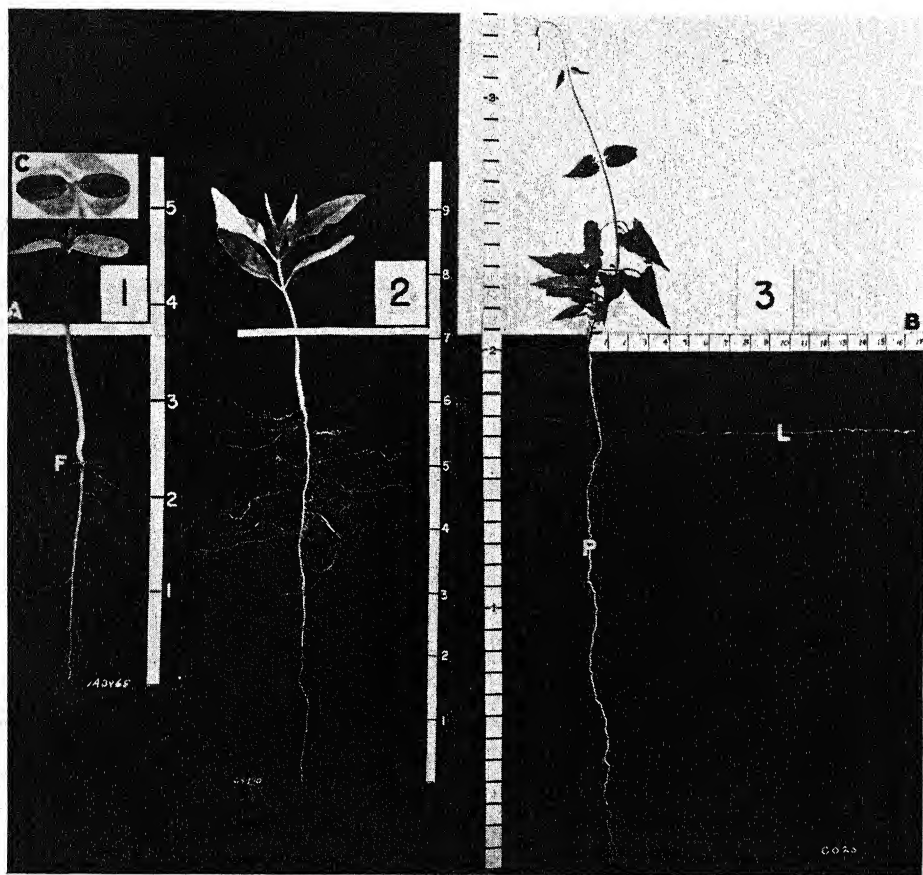
The rate of plant development under known soil and climatic conditions was observed by means of seven plants excavated 1, 2, 3, 5, 9, 13, and 25 weeks after emergence. The dates of excavation were May 26, June 2, June 9, June 23, July 21, August 18, and November 10, 1944. The plants are designated 1-7, inclusive. Figures 1, 2, 3, 4, and 6 show the vertical penetration and radial spread of roots of representative plants. Data on the plants are given in table 2.

By 5 weeks after emergence the gen-

eral plan of the root system was becoming established (fig. 3). Plant no. 6, taken the thirteenth week after emergence (fig. 4), showed particularly well the gross morphological nature of the root system. A taproot rapidly penetrated directly downward from the germinating seed. It was a primary root in order of development and vertical in position; hence, it is designated the primary vertical root (fig. 4*P*). Many branch roots arose throughout the length of this taproot, most of them small feeding roots. A few, probably those more favorably situated in relation to soil moisture and plant nutrients, grew extensively and became permanent laterals (fig. 4*L*). They are designated permanent lateral roots of the first order. These roots tended to grow as radii about the primary vertical root in a plane parallel to the soil surface in the upper 15 inches (more commonly the upper 6 inches) of the soil. They gave rise to branch roots over any part of their growth. Most of them were small feeding roots. Some, however, developed into branch lateral roots, which are designated as permanent lateral roots of the second order (fig. 4*H*). They grew horizontally, at right angles to the root on which they arose, for a distance of approximately 1-5 inches, then the majority grew horizontally away from the primary vertical root. In a similar manner permanent lateral roots of the third order arose on those of the second, and those of the fourth order on those of the third, etc. This branching tended to develop in the area between the permanent laterals of the first order. The result was that the plant occupied a much higher proportion of an ever-increasing area, somewhat circular in shape, than it would have done otherwise. The permanent laterals of the first order outgrew those of the other orders, however, so that they ex-

tended a greater horizontal distance from the primary vertical root. Concurrent studies indicated that plants subject to severe competition made less rapid horizontal development.

arose on the sides, and rarely on the top, of the permanent lateral roots of any order, in which case they grew horizontally 1-7 inches and then bent downward to become vertical taproots (fig. 6*T*). Both



FIGS. 1-3.—Development of plants of climbing milkweed. *A-B*, ground line common to all three figures. Fig. 1, seedling 1 week after emergence; *C*, view of cotyledons from above; *F*, root-stem transition zone. Scale in inches. Fig. 2, young plant 3 weeks after emergence showing two pairs of true leaves above cotyledons. Scale in inches. Fig. 3, young plant 5 weeks after emergence showing characteristic development of nutating stem which has commenced to twine. Note typical size and shape of leaves and relative length of internodes. *P*, primary vertical root; *L*, permanent lateral root. Vertical scale in feet and inches; horizontal scale in inches.

In addition to the primary vertical root (figs. 4*P*, 6*P*), other vertical roots were formed. These usually arose on the lower side of a permanent lateral of any order, from which they grew directly downward (fig. 6*S*). Less commonly they

types are designated secondary vertical roots. While these vertical roots arose on permanent laterals of any order, they naturally arose first on permanent lateral roots of the earlier-formed orders.

All shoot development, except that

from the plumule, was derived from root-borne buds. In the one growing season of 25 weeks, three root-borne buds were produced on permanent lateral roots of the third order, nine on those of the second order, and seventeen on those of the first order. None was produced on the secondary vertical roots of plants not over 25 weeks old, but they were found on all primary verticals observed of that age. The number of these buds in the

to rhizomes, but these had not emerged (fig. 4*R*). Figure 5 shows in some detail the rhizome shown at *R* in figure 4. Nodes are not readily distinguishable on the rhizomes of climbing milkweed as they are on those of field bindweed, hoary cress, Russian knapweed, and dogbane, because the small evanescent scale leaves are soon lost and the buds at the nodes, if present, are not prominent. Emergence of rhizomes first occurred 15

TABLE 2

RATE AND NATURE OF DEVELOPMENT,* AFTER SEEDLING EMERGENCE, OF ROOTS OF CLIMBING MILKWEED PLANTS, MANHATTAN, KANSAS, 1944

Plant no.	Weeks after emergence	Maximum vertical penetration (inches)	Maximum radial spread (inches)	Plant condition
1.....	1	3½	5/8	Typically shaped cotyledons (fig. 1)
2.....	2	5½	1¼	First true leaves
3.....	3	7	2 5/8	Second pair of true leaves (fig. 2)
4.....	5	10½	6½	Stem axis beginning to elongate
5.....	9	24¾	16	Later-formed internodes greatly elongated, forming characteristic nutating stem; latter, which was 16 inches long, had begun to twine. Permanent lateral roots well established (fig. 3)
6.....	13	36½	44½	Main stem 42 inches long and well supplied with leaves. Root system well established. Two well-formed rhizomes on one permanent lateral root (fig. 4)
7.....	25	42	119	Main stem 96 inches long bearing 16 fruits. Permanent lateral root of first order extending greatest distance from primary vertical root had produced 9 rhizomes, each of which bore a shoot, 8 secondary vertical roots, and 2 lateral roots of second order which branched further (fig. 6)

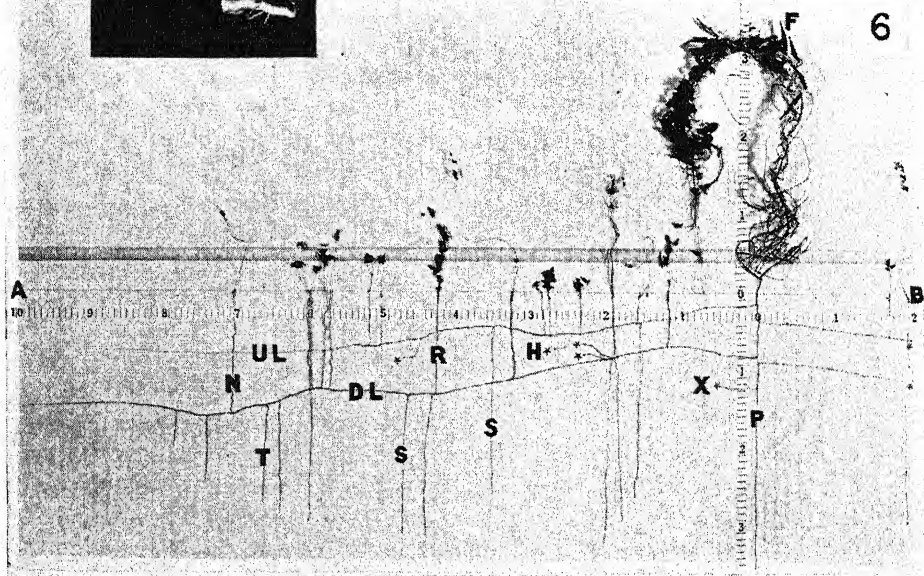
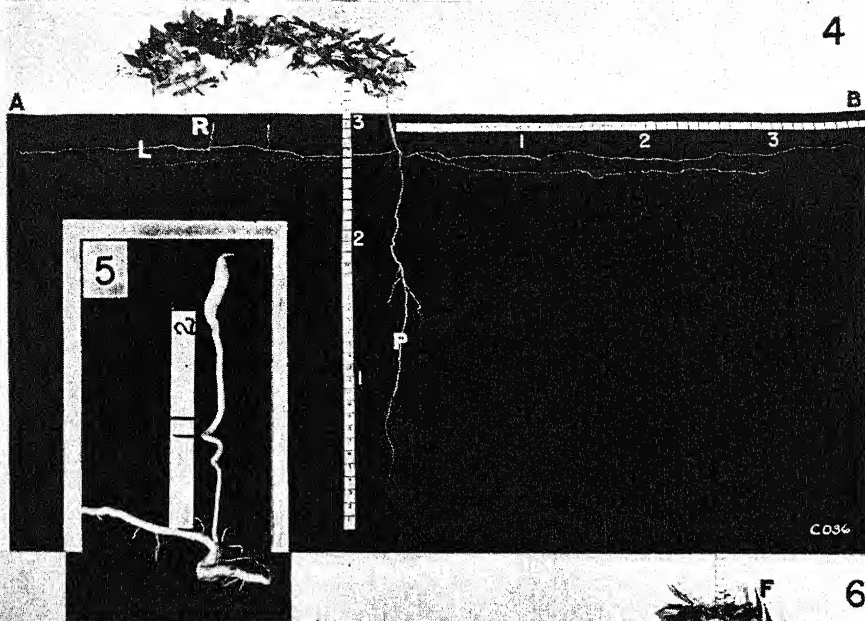
* First flowering occurred July 29, 1944.

surface 15 inches of such roots ranged from four to thirty-one. The latter appeared to be an exceptional case, and the primary vertical root in this instance had but two poorly developed permanent laterals of the first order, one of which did not exceed 2½ feet in length while the longer attained approximately 5 feet.

By 10 weeks after seedling emergence, several of these buds had been formed on the permanent lateral roots of the first order 12-18 inches from the primary vertical root. Thirteen weeks after seedling emergence such buds had given rise

weeks after seedling emergence. The above-ground portion of a rhizome is a leafy shoot. The root-borne stem buds gave rise to rhizomes unless they were borne at the ground line, in which case they gave rise to leafy shoots.

Twenty-five weeks after seedling emergence, a plant (fig. 6) had sent its primary vertical root (*P*) to a depth of 42 inches and had produced four permanent lateral roots of the first order which were arranged radially around the primary vertical. Two of these permanent laterals of the first order had formed



FIGS. 4-6.—Plant parts arranged to show essentially same relationship as in the soil. *A-B*, ground line. Fig. 4, complete plant 13 weeks after seedling emergence: *P*, primary vertical root; *L*, permanent lateral root of first order; *R*, rhizome. It had grown approximately two-thirds the distance to the soil surface. Scale in feet and inches. Fig. 5, same rhizome reversed and enlarged. Bend in lateral root has no relation to rhizome formation. Note gradual increase in size from point of origin on lateral root ending in enlargement near tip. Scale in inches. Fig. 6, portion of a plant 25 weeks after seedling emergence. Primary vertical root (*P*) subtending extensively developed shoot; portions of two permanent lateral roots to right of that root; and approximately 80% of root and shoot development of the two longest, typical permanent lateral roots of the first order. The one (*UL*) developed in soil that had not been disturbed in recent years; the other (*DL*) grew 4 feet into soil which had been removed to a depth of 2 feet 2 years earlier, then replaced (each foot level in proper place) at that time. *S*, secondary vertical root of type more commonly found, originating on lower side of permanent lateral root; *T*, secondary vertical root of less commonly found type originating on side of permanent lateral root; *H*, permanent lateral root of second order (there are two on *UL* and two on *DL*); *X*, stub of a permanent lateral root of first order; *N*, older rhizome showing development at that stage; *F*, fruit. Stars indicate points where roots have been removed for clarity. Many leaves, particularly on shoots of the older rhizomes, were lost before picture was taken. Scale in feet and inches.

permanent laterals of the second and higher orders. One had a permanent lateral of the fourth order. Three of the permanent lateral roots of the first order grew in an undisturbed soil comparable with that of the other studies of this series (2, 3, 4, 5). The lateral root (fig. 6UL) is one of these three permanent laterals that grew in undisturbed soil. It had six rhizomes, each of which bore a shoot, two secondary vertical roots, and two permanent lateral roots of the second order, one of which had a permanent lateral root of the third order. The permanent lateral root (fig. 6DL) extended 4 feet of its total length of 10 feet into soil which had been excavated to a depth of 2 feet during root studies made 2 years earlier, then replaced—each foot level in place—at that time. This permanent lateral root was somewhat more extensively developed. It had nine rhizomes, each of which bore a shoot, eight secondary vertical roots, and two permanent lateral roots of the second order, of which both had permanent laterals of the third order and one had a permanent lateral of the fourth order.

Discussion

The rate of growth of plants of climbing milkweed from seed under known soil and climatic conditions, with little or no competition, is not to be construed as representing their growth rate under all conditions. It illustrates only the growth potential in a favorable noncompetitive situation.

HITCHCOCK and CLOTHIER (7), in a study on this weed, which they called *Enslenia albida* Nutt., observed a vertical root which extended 7 feet deep. It was from a plant of unknown age growing in the field at Manhattan, Kansas. BRUNS (1) traced vertical roots of climbing milkweed plants of unknown age growing in the field near Canton, Kansas,

to a depth of 6½ feet. None extended below that depth, although roots of field bindweed, *Convolvulus arvensis*, immediately adjacent were excavated to a depth of 12 feet without reaching the tip. The compacted soil layers at depths of 26 and 32 inches at the site of the study here reported may have influenced vertical root penetration.

BRUNS (1) did not find permanent lateral roots on the plants of climbing milkweed he studied. He believed that permanent lateral roots, if present, were located so near the surface that they were destroyed by a plowing 6 inches in depth that had been made on the area. HITCHCOCK and CLOTHIER (7) used permanent lateral roots from an average depth of 10 inches below the surface for regeneration studies. These lateral roots were obtained from field-grown plants of unknown age. In some of the plants observed in the study reported here all the permanent lateral roots of the first order were located in the surface 6 inches of the soil (fig. 4); in other plants many were below that depth (fig. 6).

Research to determine why certain of the branch roots develop more extensively than others and become permanent lateral roots has not been undertaken. It has been conjectured by KENNEDY and CRAFTS (9) for field bindweed, and by FRAZIER (3, 4, 5) for hoary cress, Russian knapweed, and dogbane, that these variations result from differences in the supplies of soil moisture and plant nutrients.

The nature of development and role of the permanent lateral roots of the first order of climbing milkweed are the same as found for dogbane by FRAZIER (5). In both plants these roots grow approximately horizontally if variations in slope, soil texture, and moisture supply are taken into consideration. In no instance were the growing tips of these

roots observed to turn downward and thus become vertical roots, as described for field bindweed, hoary cress, and Russian knapweed (2, 3, 4).

HITCHCOCK and CLOTHIER (7) in 1898 observed in a general way the nature and extent of the horizontal growth of the underground lateral axes of climbing milkweed and recognized these axes as roots rather than as stems. They observed, as was confirmed in the present study, that there was no regularity in the position of the root-borne buds on the lateral roots, but apparently they did not realize the nature of the secondary vertical roots and their relation to the permanent laterals.

The rhizome of climbing milkweed, especially the tip, is fragile and easily damaged. It grows readily through a moist friable soil but has difficulty emerging in a dry soil that has formed a crust. The young rhizome is quite small in diameter near its place of origin (fig. 5), but once it has formed a shoot it may increase rapidly, particularly in a loose soil, to become a much-thickened structure (fig. 6*N*). In striking contrast to field bindweed, which under favorable conditions of growth forms a number of new rhizomes in 5-8 days after the destruction of the existing rhizome, climbing milkweed usually forms but one rhizome for each one destroyed, and emergence requires 14-22 days—even under favorable conditions.

Summary

1. Plants of climbing milkweed, grown from seed on a typical upland loam soil at Manhattan, Kansas, under known temperature and precipitation conditions and not subject to competition, were studied at various ages, from the seedling stage through 25 weeks of growth, to determine the nature and rate of development.

2. The root system of well-established plants consisted of the original root (primary vertical) and one to many permanent laterals which continued to grow horizontally and on which arose roots that grew either downward directly, or did so after short horizontal growth, to become secondary vertical roots.

3. The plants spread horizontally by means of the permanent lateral roots. The permanent laterals of the first order arose on the primary vertical root. Branch lateral roots (laterals of the second order) arose on the permanent laterals of the first order. In a similar manner permanent laterals of the third order arose on those of the second, and those of the fourth order arose on those of the third, etc. Concurrent studies indicated that injury or too severe competition prevents extensive horizontal growth of the laterals of any order.

4. The plants spread radially 9 feet in undisturbed soil (10 feet where the last 4 feet penetrated soil which had been excavated and replaced two years earlier) and reached a depth of $3\frac{1}{2}$ feet in a growing season of 25 weeks.

5. The source of shoot development, other than that arising from the plumule, was from root-borne buds which produced shoots directly (if at the ground line), or rhizomes (if below ground) which in turn gave rise to leafy shoots. There was no regularity in the location of these adventitious buds on the permanent lateral roots. None of these buds was observed on secondary vertical roots. The shoot development of old plants was wholly from root-borne buds.

6. The type of development is similar to that of dogbane, and their common type of development exhibits certain similarities to, and certain differences from, the type common to field bindweed, hoary cress, and Russian knapweed.

weed. The horizontal spread in both types is by means of permanent lateral roots, but in the dogbane-climbing milkweed type the lateral roots continue to grow horizontally and do not bend downward to produce vertical roots (secondary verticals) as they do in the other type. In the dogbane-climbing milkweed type, secondary vertical roots arise on the

permanent lateral roots. The development of shoots appears to be the same in both types. Field bindweed and climbing milkweed were the only two of the five species to flower and fruit during the first growing season.

DEPARTMENT OF BOTANY
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MANHATTAN, KANSAS

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SECOND-YEAR DEVELOPMENT OF ROOT SYSTEM OF APOCYNUM CANNABINUM

The nature and rate of development of the root system of this weed, commonly known as dogbane, reported for the first growing season (28 weeks of growth) (BOT. GAZ. 105:463-470. 1944), have now been studied through the second growing season. The soil profile was further described to a depth of 14 feet by Dr. J. C. HIDE of the Kansas Agricultural Experiment Station. The profile description for the 7-14-foot interval was similar in texture and structure to the 5-7-foot interval described in the article cited, except that it became slightly more friable with depth. The plant most extensively developed in 77 weeks following seedling emer-

gence on May 17, 1943 (from a planting made May 3, 1943), was excavated November 6, 1944. In the two growing seasons its primary vertical root attained a maximum vertical penetration of 13 feet, 8 inches, the deepest secondary vertical root 7 feet, 9 inches, and the most extensively developed permanent lateral root of the first order reached 19 feet, 6 inches, in length. The first flowering of this plant occurred May 25, 1944, and many fruits were matured that year (1944).—JOHN C. FRAZIER, *Contribution no. 468; Department of Botany, Kansas Agricultural Experiment Station, Manhattan, Kansas.*

INVERSE CORRELATION BETWEEN RUBBER HYDROCARBON AND A CRYSTALLINE FRACTION ISOLATED FROM LATEX OF *CRYPTOSTEGIA GRANDIFLORA*

WILLIAM S. STEWART¹ AND RICHARD W. HUMMER²

Introduction

A crystalline fraction has been isolated from dried latex of *Cryptostegia grandiflora*. Available data indicate that there is an inverse correlation between this crystalline fraction and the rubber hydrocarbon in the latex. This relation may afford an opening wedge into the problem of rubber formation in plants.

C. grandiflora, a member of the Asclepiadaceae, is one of several plants being studied as an emergency source of rubber. The plant is either a large shrub or a vine, depending on available support for vining. In addition to foliage branches, it produces relatively long, rapidly growing, mostly unbranched leaders which tend to twine around any available support. These leaders, or whips, as they sometimes are called, usually originate from lateral buds along the thicker main stems which form the body of the shrub. Their diameter near the tip is 2-3 mm., widening to 15-20 mm. near the base. For nearly one-third of their length from the tip they are either completely leafless or the small unexpanded leaves are adnate to the whip. In this region the whips usually are succulent, very slightly lignified, and have a large proportion of pith and cortex, whereas the lower part of the whip is woody, lignified, and with proportionally little pith. A more complete description of the plant is given by POLHAMUS *et al.* (4). Failing to twine on a support, the whip

may grow to a length of 12-15 feet before bending downward from its own weight. As the whip continues to grow and bend, short, leafy, lateral branches are produced and it becomes increasingly woody. The weight of these branches in turn contributes to the further bending of the whip down within the body of the shrub. New whips, which often originate from old foliated ones, are continually produced and replace the older ones. Cutting off the tips of the whips before they bend downward and collecting the exuding latex has been one suggested method of utilizing the plant as an emergency source of rubber. This general procedure, called "whip bleeding," was the manner in which most of the latex samples used in this investigation were collected.

Method

During the course of yield determinations by whip bleeding from March, 1943, through July, 1944, 157 latex samples were collected. These were dried to constant weight in a thermostatically controlled oven at 60° C., instead of being coagulated as usual by some such agent as alcohol or water. For the studies reported here, the importance of drying the latex instead of using a coagulating agent cannot be overemphasized. In common practice, after the use of a latex-coagulating agent, the serum is usually discarded and only the coagulate analyzed. While this may be satisfactory for quality determinations of the coagulate, it yields physiological information of limited value, since a portion

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of the latex is discarded with no further consideration.

The samples represented collections of extremely wide diversity. They were secured from the vicinity of Brownsville, Texas, and Ciudad Victoria, Tamaulipas, Mexico. Some of the collections were composite yields of latex from single whips on individual plants bled four to seventeen times consecutively; others were pooled yields from various amounts of successive bleedings of 20 to 50 plants. Some of the samples were from plants 7 months old; others were from plants estimated to be 15 years old. In most collections the whips were of the type just described, although a few samples were from foliage branches of small diameter ($\frac{1}{4}$ inch or less), very old bent-down whips, thick ($1\frac{1}{2}$ inch diameter) basal foliage stems, and seed pods. All collections were from the above-ground portion of the plants. Because of the extremely wide diversity of the collections, the whip internode in which the bleeding was made, the number of previous bleedings of the whip, the time interval between bleedings, the time of day of the bleeding, the season of the year, and the amount of whip cut away per bleeding were factors varying from sample to sample.

The oven-dried latex samples were analyzed for rubber hydrocarbons by a bromination technique and for insolubles by a direct gravimetric determination. These methods were kindly supplied by private communication from Dr. C. O. Willits of the Eastern Regional Research Laboratory, and are as follows.

Accurately weighed samples of the dried latex, sometimes called "total latex solids," are cut into small pieces, placed in tared centrifuge tubes, and treated with benzene containing 1% trichloroacetic acid. After standing for 3

days, with occasional stirring, insoluble substances are separated by centrifuging and washing with benzene. The benzene solution and washings are made to volume with benzene, and aliquots are brominated using a solution of bromine and iodine in carbon tetrachloride. The "rubber tetrabromide" so formed is precipitated with 95% ethyl alcohol, filtered, dried, and weighed. The percentage of rubber in the sample is calculated by multiplying the weight of rubber tetrabromide by the empirical factor of 0.285.

The substances insoluble in benzene which remain in the centrifuge tubes are thoroughly extracted several times with acetone. The insolubles still remaining are separated by centrifuging, dried at 60° C., and weighed. Insolubles prepared by this method—using trichloroacetic acid in the benzene extraction (1)—have been shown to be completely free of rubber hydrocarbons.

By these methods, "insolubles" in latex total solids are defined as substances which are insoluble in either acetone or benzene, and "rubber hydrocarbons" are defined as benzene-soluble substances which form bromides insoluble in 95% ethyl alcohol. The insolubles and rubber hydrocarbons are each directly determined, percentages being based on original weight of latex sample dried at 60° C.

Results and discussion

Analyses of the diverse assortment of latex samples indicated that they varied in such a way that the percentage of rubber hydrocarbons was, without exception, inversely correlated with the percentage of insolubles (fig. 1). The correlation coefficient, significant at the 1% level, was -0.9371 . Apparently this inverse correlation was a linear relationship. From these analyses, using the

method of least squares (5) for the computation of regression averages, it was possible to calculate the equation: $R\% = 77.8 - 0.809 I\%$, where R = rubber hydrocarbons and I = insolubles. The regression coefficient, significant at the 1% level, is -0.809 . This equation defines the regression line RI (fig. 1) which has intercepts on the rubber hydrocarbons and insolubles axes of 77.8 and 96.2, respectively, and is calculated from 149 analyses. These are theoretical limits based on extrapolation and are subject to experimental error. The standard error of the estimate was 2.6%, which represents graphically an average of the vertical distances of the dots from RI . Table 1 gives the analyses of twenty-five of the 149 samples represented in figure 1.

Analyses of twenty-three additional samples of latex dried at 60° C. were made by a direct extraction method described by HALL and GOODSPEED (2). The determined percentages of rubber hydrocarbons and insolubles for each sample were located on the calculated RI regression line. These analyses, however, were not used in computation of the regression line, nor are they shown in figure 1, since by this method of analysis the insolubles are determined indirectly. Latex samples of average resin content, analyzed by this method, might appear to have—within the limits of standard error of the regression line RI —rubber hydrocarbons and insolubles inversely correlated, solely because of incomplete extraction of rubber hydrocarbons. For this reason an inverse correlation based on analyses by this method may or may not be correct, depending on the completeness of the extractions.

To concentrate the substances present in the insolubles which caused the inverse correlation with rubber hydrocarbons, a survey was made to discover

solvents which could extract them from the "insolubles." It was found that they were dissolved by water, by 95% ethyl alcohol, and by absolute ethanol. Further trials indicated that if the insolubles were dried to a constant weight at 100° C. before extraction with ethanol, an additional separation of this extract (E) into two portions was possible by a fractional precipitation technique. The separation

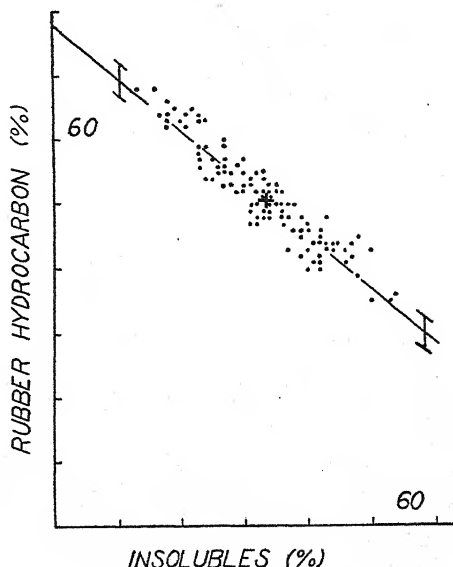


FIG. 1.—Inverse correlation between percentage of rubber hydrocarbons and percentage of insolubles in dried latex of *C. grandiflora*. Regression line, with standard error, calculated from 149 latex analyses. Cross indicates average point.

of latex insolubles into a crystalline fraction inversely correlated with rubber hydrocarbons and noncorrelated fractions is shown in the following schematic representation. Letters in parentheses identify the fraction with the corresponding discussion in the text. The first fraction was obtained by adding petroleum ether to the ethanol until an oily, brown, flocculent precipitate formed (P). Failing to form a precipitate on addition of a volume of petroleum

ether equal to that of the ethanol, the solution (*EE*) was partially evaporated at 100° C. until a precipitate formed. In some cases it was necessary—even after this evaporation—to add more petroleum ether to change the solubility relation-

ships in the solution so that a precipitate would form. After quantitatively separating the precipitate from the solution, this same treatment was repeated on the filtrate several times until no further precipitate formed. The second fraction

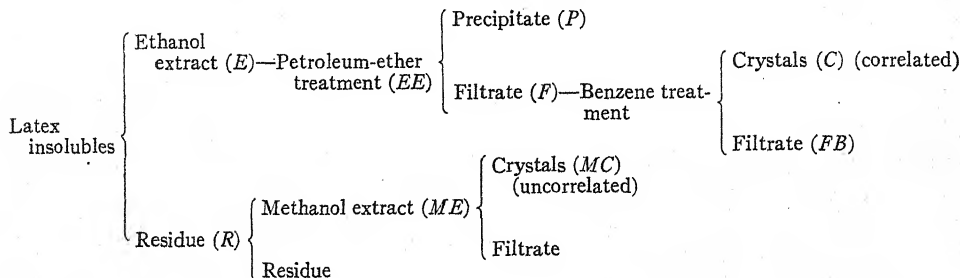


TABLE 1

NUMERICAL DATA FOR TWENTY-FIVE OF THE 149 *CRYPTOSTEGIA GRANDIFLORA* LATEX TOTAL SOLIDS ANALYSES SHOWN GRAPHICALLY IN FIGURE 1. LATEX OBTAINED BY WHIP BLEEDING UNLESS OTHERWISE INDICATED

SAMPLE NO.	LATEX SOURCE		COLLECTION DATE	LATEX ANALYSES (%)		
	Bleeding	Other data		Rubber hydrocarbons	Insolubles	Undetermined (remainder)
106.....	2nd	Whips defoliated by hand	2/25/44	35	53	12
59.....	8th	Fifteenth internode	8/28/43	36	54	10
121.....	2nd	Entire plant defoliated	2/25/44	37	45	18
37.....	16th	At 24-hr. intervals	8/29/43	39	48	13
99.....	7th	Entire plant defoliated	2/5/44	40	40	20
116.....	1st	Whips 2-3 ft. long	3/9/44	41	42	17
135.....	1st	Plants in 5-gal. containers	6/2/44	42	42	16
159.....	4th	At 24-hr. intervals, 6:00 P.M.	3/23/44	43	37	20
169.....	5th	Plants in 5-gal. containers	6/7/44	44	41	15
113.....	18th	At 48-hr. intervals	3/24/44	45	39	16
146.....	1st	2:00 P.M.	3/20/44	46	38	16
11.....	10th	At 72-hr. intervals	9/10/43	47	36	17
26.....	6th	At 24-hr. intervals	8/19/43	48	34	18
148.....	2nd	At 24-hr. intervals, 6:30 A.M.	3/21/44	49	33	18
23.....	4th	At 24-hr. intervals	8/17/43	50	35	15
151.....	2nd	At 24-hr. intervals, 6:00 P.M.	3/21/44	51	34	15
131.....	1st	Sixth internode	5/11/44	52	32	16
2.....	2nd	Eighth internode	8/15/43	53	34	13
145.....	1st	Third internode, whips 10 ft. long	6/15/44	54	31	15
144.....	1st	Third internode, whips 6 ft. long	6/15/44	56	27	17
28.....	6th	At 24-hr. intervals	8/19/43	58	23	19
166.....		Seed pods, nearly mature	3/12/44	59	23	18
101.....	8th	At 48-hr. intervals	8/28/43	63	18	19
109.....	14th	At 48-hr. intervals, two bleedings per internode	9/9/43	65	19	16
110.....	14th	At 48-hr. intervals, one bleeding per internode	9/9/43	68	13	19

was then obtained by adding benzene to the filtrate (*F*) until the formation of crystalline platelets began (*C*). Here, as with the addition of petroleum ether, if no crystallization began after the addition of a volume of benzene equal to that of the filtrate, it was necessary to evaporate the solution until crystallization began and in some cases to add more benzene. These manipulations were repeated until no further crystal formation was obtained. Evaporation of the remaining filtrate (*FB*) yielded a residue which was less than 1% of the original sample. This was considered negligible.

The ethanol extract (*E*) of insolubles from latex samples low in rubber hydrocarbons often was dark reddish brown, while from those high in rubber hydrocarbons it was straw-yellow color. After complete precipitation of the brown precipitate (*P*) by the addition of petroleum ether, the filtrate (*F*) was usually light yellow. This color change was indicative of the completion of precipitation. If the precipitation was not complete, crystals (*C*) contaminated with a slight amount of brown color resulted. This was removed by redissolving the crystals in a small volume of hot ethanol, adding powdered activated charcoal to adsorb the brown color, filtering, and then repeating the crystallization procedure. A loss of about 40% usually occurred from this treatment, thus making it unsatisfactory for a quantitative determination of the crystals.

Identification of the crystals (*C*) from the ethanol extract is not yet complete, but a few properties have been determined. They are white, slightly bitter-tasting, and strongly birefringent. They begin to char at 180° C. and melt between 234° and 236° C. Crystals isolated from the serum of auto-coagulated latex of *C. grandiflora* have been reported to

have a bitter taste and to melt at 236–7° C. (3), and so they may be the same as those described here.

To determine the extent to which either the flocculent precipitate (*P*) or the crystalline fraction (*C*) might be inversely correlated with rubber hydrocarbons, eight latex samples of known composition were selected. Aliquots of these were re-analyzed to check the previous determinations. These samples varied in percentage of rubber hydrocarbons from 38 to 62 and in percentage of insolubles from 47 to 19. Their selection thus represented a relatively wide distribution along the rubber hydrocarbons-insolubles regression line. The insolubles obtained by the analytical method from these samples were extracted by using four portions of absolute ethanol with stirring. After extraction, the residue was washed with an additional small portion of absolute ethanol and the extracts and washings combined for each sample. These were then separated into crystalline (*C*) and noncrystalline (*P*) fractions by the technique described. The crystalline fraction was inversely correlated with rubber hydrocarbons, but the noncrystalline portion was not correlated (fig. 2). The coefficients of correlation for rubber hydrocarbons and crystals, and for rubber hydrocarbons and noncrystalline portion, were -0.9056 and -0.5753 , respectively. The correlation coefficient for crystals was significant at 1%, but that for the noncrystalline portion lacked significance at 5%. The equations with standard error of the estimate, for the regression of percentage rubber hydrocarbons on percentage of insolubles, ethanol extract (*E*), and crystals (*C*), as given in figure 2, are: $R = 75.5 - 0.780 I, \pm 2.6$; $R = 70.0 - 1.025 E, \pm 3.2$; and $R = 68.8 - 1.218 C, \pm 3.8$, respectively. The equa-

tion for the regression of percentage precipitate (P) on percentage rubber hydrocarbons is: $R = 86.4 - 11.4 P$, ± 1.1 . This experiment was repeated on three additional latex samples with similar results.

While the crystalline fraction appears to account for the major portion of the observed inverse correlation between rubber hydrocarbons and insolubles,

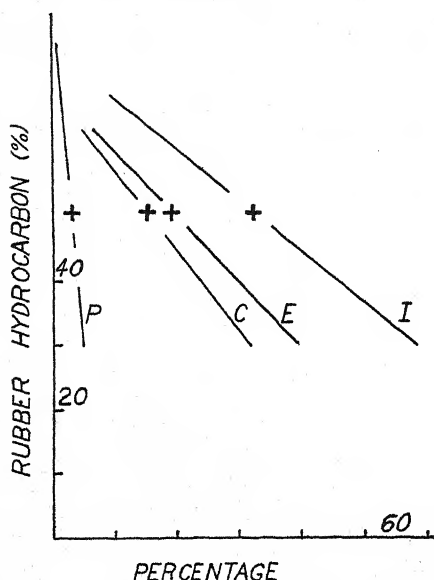


FIG. 2.—Inverse correlation in dried latex of *C. grandiflora* between percentage of rubber hydrocarbons and percentage of insolubles, regression line I ; ethanol extract of insolubles, line E ; and crystalline fraction from ethanol extract, line C . Crosses indicate average points in each case. Correlation coefficients for these regressions are -0.9557 , -0.9337 , and -0.9056 , respectively (significant at 1%). Precipitate from ethanol extract (line P) not significantly inversely correlated with rubber hydrocarbons.

whether or not it alone accounts for the entire correlation is not known—since an inverse correlation was observed between rubber hydrocarbons and the insolubles residue (R) left after ethanol extraction. The correlation coefficient was -0.7537 , significant at 5%. The regression of rubber hydrocarbons on this residue is

$73.6 - 1.779 IR$, where IR equals the insolubles not extracted by ethanol. Standard error of the estimate is ± 5.09 . The regression coefficient is also significant at 5%.

Since the correlation between rubber hydrocarbons and insolubles residue (R) could have resulted from incomplete ethanol extraction of the insolubles, more complete extractions were made by shaking the insolubles overnight in ethanol, following three 10-minute extractions with hot ethanol. These were carried out on the insolubles from three large lots of latex which were being used to prepare a quantity of crystals. The overnight shaking extraction was repeated two additional times on new aliquots of insolubles from the same three lots, which had percentages of rubber hydrocarbons and insolubles of 37, 33; 45, 24; and 56, 12; respectively. The crystalline fraction (C) was obtained from the extract by the fractional precipitation technique described. As in the previous experiment, the crystalline fraction (C) accounted for the correlation of the extract (E), but in every trial the extract still apparently failed to contain all the correlated portion of the insolubles. The insolubles residue (R) remaining after ethanol extraction still had a very slight but significant inverse correlation with rubber hydrocarbons. Owing to mechanical difficulties, because of the large size of the lots, recovery of the insolubles from the oven-dried latex was incomplete. This located the regression line of rubber hydrocarbons on latex insolubles (fig. 3) slightly toward the left of the original line given in figure 1. The slope of this line is the same as that of the original, however, since the determinations of rubber hydrocarbons were not changed by the size of the lots.

Further investigation of this appar-

ently correlated residue (*R*) indicated that it was possible to separate it further by extraction with absolute methanol. From duplicate methanol extractions of the residue (*R*) from the three lots of latex, it was found that this extract (*ME*) was correlated with rubber hydrocarbons. After extraction, crystal formation was readily induced by reducing the volume of the extract. These crystals (*MC*), like the methanol extract (*ME*), were correlated with rubber hydrocarbons. Their properties and identity are being determined. It was clear, however, that these crystals differed from those obtained by the ethanol extraction, since these lacked birefringence. They were readily soluble in methanol and also very slightly soluble in ethanol. Because of their ethanol solubility, the amount of ethanol extract (*E*) from insolubles will depend in part on the amount of these noncorrelated crystals (*MC*) present in the insolubles.

A further attempt was made to ethanol-extract all the correlated substances from the insolubles by using micro-Soxhlet extractors. Three insolubles samples of 200 mg. each were extracted 48 hours. The extraction was complete, as indicated by constant weight of the residue during the final 12 hours. During the extraction, crystals precipitated from the ethanol. These crystals lacked birefringence, were very readily soluble in methanol, and appeared to be the same as those just described (*MC*). They amounted to 12.0, 2.9, and 5.4 mg. from the three latex samples which had 56, 45, and 37% rubber hydrocarbons, respectively. In this way the ethanol extract (*E*) of insolubles from samples highest in rubber hydrocarbons was increased by this noncorrelated portion over those from lower percentages of rubber hydrocarbons. Since some of these

noncorrelated crystals (*MC*) probably also remained in the ethanol solution, when considered together with those which precipitated out, they could probably account for the convergence of the two regression lines—rubber hydrocarbons on insolubles and rubber hydrocarbons on extract—at the high percentage levels of rubber hydrocarbons (figs. 2, 3).

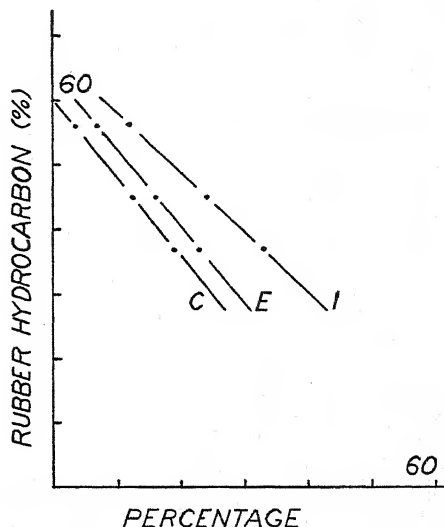


FIG. 3.—Inverse correlation in dried latex between percentage rubber hydrocarbons and percentage of insolubles (*I*); ethanol extract of insolubles (*E*); and crystalline fraction from ethanol extract (*C*).

Methods are being investigated for more completely separating the two crystalline fractions (*C* and *MC*) found in the insolubles. While it seems possible that the crystalline fraction (*C*) from the ethanol extract may be the only one in the latex inversely correlated with rubber hydrocarbons, conclusive proof will await the development of its more selective isolation from the insolubles.

When fresh latex is dried on a sheet of glass, both the correlated ethanol fraction crystals (*C*) and the apparently

noncorrelated "methanol" crystals (*MC*) are visible. The former may be seen readily with the naked eye, but the latter are usually apparent only on microscopic examination. That the "ethanol" crystals (*C*) are readily visible in latex dried in this fashion is not surprising, since they constitute 19% of the total latex solids from latex which has 37% rubber hydrocarbons.

The significance of the inverse correlation between rubber hydrocarbons and the crystalline fraction (*C*) extracted from the insolubles by ethanol is not known. The crystalline substance might be directly converted into rubber hydrocarbons within the plant and so be considered a "precursor" of rubber hydrocarbons; but it is equally possible that it might be a substance very indirectly related to rubber hydrocarbons. For example, it might be derived from the same source of materials as rubber hydrocarbons, and so in effect be indirectly re-

lated to them by competition for its origin. Identification of the crystals and physiological experiments in progress may indicate the role this substance has in rubber formation and latex physiology.

Summary

1. Analysis of 157 samples of dried latex from above-ground portions of *Cryptostegia grandiflora* indicated the existence of a linear inverse correlation between the percentage of rubber hydrocarbons and the insolubles.

2. By extraction of the latex "insolubles" with absolute ethanol and fractional precipitation of the extract, an unidentified crystalline fraction was obtained which was inversely correlated with rubber hydrocarbons. It is possible that this crystalline fraction is the only one in the insolubles and latex inversely correlated with the rubber hydrocarbons.

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RUBBER CONTENT, STEM ANATOMY, AND SEED PRODUCTION AS RELATED TO RATE OF VEGETATIVE GROWTH IN GUAYULE

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Introduction

LLOYD (4) observed that, under natural conditions, rubber accumulated most readily in plants of guayule (*Parthenium argentatum* Gray) during those periods of the year least favorable to vegetative growth. BONNER (3) reported that, under experimental conditions, guayule plants attained a higher concentration of rubber in their stems and roots when their vegetative growth was retarded to some extent by subjecting them to night temperatures of 35°–50° F. than when they were subjected to temperature conditions that favored continuous growth. These results suggest that rubber storage in guayule occurs mainly during the winter season, when the rate of vegetative growth is at a minimum.

In the present experiments, plants were grown which differed widely as to succulence and amount of vegetative growth produced during the summer growing season. These differences were secured by varying the amount of nitrogen, and—in some instances—the amount of calcium and nitrogen, supplied to the plants. The plants were studied in regard to (a) their relative efficiency in producing rubber, (b) the anatomical characteristics of their stems with respect to their capacity for rubber storage, and (c) the number and quality of seeds produced.

Methods

Seedlings (strain 593) that had been grown for one season under field conditions at an Emergency Rubber Project nursery near Salinas, California, were selected for size and uniformity. They were planted in a mixture of coarse sand and fine gravel contained in 3-gallon glazed earthenware crocks provided with adequate drainage. Two experiments are reported here.

In preparing a basic nutrient solution for the first experiment, sufficient CaCl_2 was added to tap water to make a concentration of 17.5 milliequivalents of calcium per liter and sufficient KH_2PO_4 and MgSO_4 to make a concentration of 2.6 me. of the respective ions per liter of nutrient. Sufficient ferric citrate, manganese chloride, cupric chloride, zinc chloride, and boric acid were added to make 0.3, 0.25, 1.0, 1.0, and 1.5 p.p.m. of Fe, Mn, Cu, Zn, and B, respectively. Treatments consisted of five nutrient solutions made by adding sufficient NaNO_3 to the basic solution to make 1.4, 3.6, 7.2, 14.4, and 28.8 me. of NO_3 ion, respectively.

The pots, each containing one plant, were arranged on a gravel bed in successive rows of four plants each. The experimental design consisted of randomized blocks with sixteen plants per treatment and four replications of each treatment (fig. 1). Approximately 400 ml. of nutrient solution was applied to each pot three times weekly, an amount sufficient to flush the gravel thoroughly. Tap water was used to maintain moisture supply between nutrient treatments. The plants

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were grown out-of-doors from July to October, 1943, then arranged in the same order in a greenhouse, where temperatures of 35°-45° F. were maintained during the night and 60°-70° F. during the day. In February, 1944, all plants were harvested, weight and anatomical measurements were made, and the rubber and resin contents of the roots and stems were determined by the SPENCE and CALDWELL method (7).

In the second experiment, selected plants from the same shipment of seed-

and five replications, as previously described. The experiment was started during the first week of June, 1943. Plants of treatments 1, 2, 3, and 4 received nutrient containing 1.8, 7.2, 14.4, and 21.5 me. of calcium and nitrate ions per liter, respectively, the treatments being continued until the plants were harvested in February, 1944. Plants of treatment 5 received 1.8 me. of calcium and nitrate ions during approximately the first half of the growing season (July 21, 1943), then 21.5 me. during the remainder of the growing

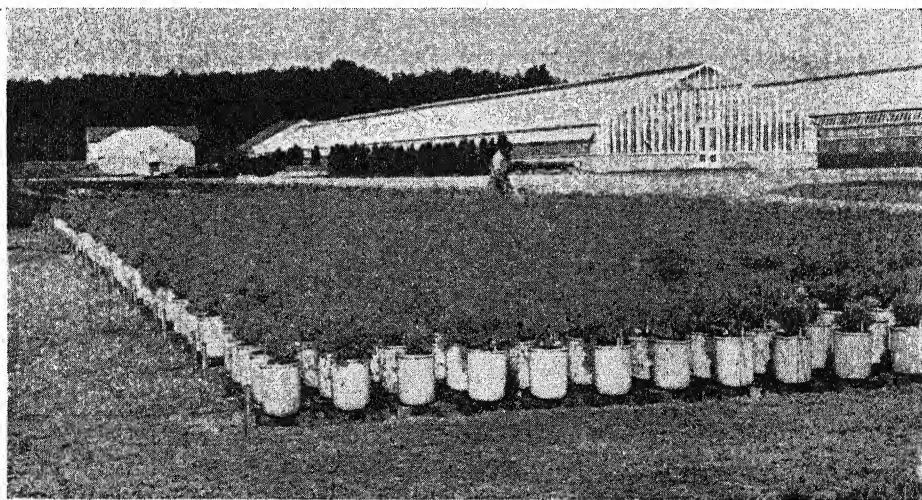


FIG. 1.—Field arrangement of guayule plants and greenhouses in which the plants were grown during the winter months under controlled temperatures.

lings were grown in a similar manner. The basic nutrient used contained 2.6 me. of K, Mg, H_2PO_4 , and SO_4 ions per liter and the same concentrations of microelements as previously described. The concentration of calcium and nitrate ions was varied from 1.8 to 7.2, 14.4, and 21.5 me. per liter by adding desired amounts of $Ca(NO_3)_2$.

The plants were grown out-of-doors, arranged in rows of five plants each. Treatments were applied as randomized blocks with eight variations of nutrient

season (which ended the latter part of September) and winter dormancy period. Plants of treatment 6 received 7.2 me. and then were changed in a similar way to 14.4 me. per liter; those of treatment 7 received 14.4 me. and later 7.2 me.; while those of treatment 8 were supplied with nutrient containing 21.5 me. of calcium and nitrate ions per liter for the first half of the growing season and then shifted (on July 21, 1943) to a solution that contained 1.8 me. In this way the growth of some plants was limited by nu-

trient treatments during the entire experiment, while others supplied with adequate nutrients grew vigorously during the summer and fall, their growth being checked by low temperatures during the winter months. Some were made to grow slowly during the first part of the summer and then rapidly during the remainder of the growing season, while others grew rapidly at first and then more slowly during the latter part of the growing season. All plants were moved to a greenhouse in October and subjected to the same light and temperature conditions throughout the winter dormancy period. Nutrient treatments begun during summer were continued unchanged throughout the fall and winter seasons.

Seeds were collected weekly during four periods: from June 1 to July 15, July 15 to August 9, August 9 to September 1, and September 1 to September 16. They were cleaned and stored at room temperature during the following winter. Later they were sorted for size, and tested for germination as described previously (6).

At the time of final harvest (February, 1944), material for a limited anatomical survey was collected. Of those plants grown with variation in the calcium and nitrogen supply, samples were selected from each of three plants from each of four levels of nutrition: 1.8, 21.5, 1.8 changing to 21.5, and 21.5 changing to 1.8 me. of nitrate ion per liter, with the accompanying changes in calcium. One series of samples consisted of segments 1 cm. in length taken from the main stem of each plant in the region just above the cotyledonary node. The extent of topping previous to transplanting and the consequent low branching of the plant caused these segments to be highly irregular in form and difficult for anatomical measurements. Accordingly, a sec-

ond series of samples from each plant was secured from the base of a major branch representative in size for the general growth of the plant. Of those plants grown with varying amounts of nitrogen, similar samples were selected from the base of each of two average branches of three plants each from the five levels of nitrogen nutrition. The methods used in preparing slides and determining cross-sectional areas of wood, bark, and fiber tissue in the bark have been described previously (8).

The term "significant" is used in this paper to mean that differences between means were significantly different by odds of 19:1.

Results

RESPONSES TO VARIATIONS IN NITROGEN SUPPLY.—An increase in nitrogen supply from 1.4 to 14.4 me. per liter of nutrient resulted in marked increase in vegetative growth, as reflected in the dry weight of stem and roots; further increase in nitrogen supply up to 28.8 me. did not significantly increase the growth of stems and roots as measured by their dry weight (table 1). In contrast, the percentage of rubber in the stems and roots was not influenced by these extreme variations in the amount of nitrogen supplied to the plant. As a result, the total rubber produced per plant paralleled the amount of its vegetative growth.

Increasing the nitrogen supply from 1.4 to 14.4 me. was associated with a significant increase in the cross-sectional area of the branches (table 2); increase above this amount, up to 28.8 me., resulted in no further significant increase in diameter. The percentage of bark in stems of plants that were stunted owing to the limited amounts of nitrogen supplied was greater than that of plants which received increased amounts of ni-

trogen and grew vigorously. However, this difference was not wholly due to the nutrient treatments, but was largely due to inherent differences in proportions of tissues in small and large stems. This

TABLE 1

EFFECT OF VARYING THE AMOUNT OF NITRATE ION IN THE NUTRIENT ON GROWTH AND AMOUNT OF RUBBER PRODUCED BY GUAYULE. EIGHT MONTHS AFTER TRANSPLANTING

Nitrogen per liter of nutrient (me.)	Average fresh weight per plant (gm.)	Average dry weight of stems and roots (gm.)	Average percentage rubber in stems and roots (%)	Average rubber per plant (gm.)
1.4.....	38.7	9.1	4.99	0.45
3.6.....	66.4	14.4	4.99	0.72
7.2.....	105.1	21.8	4.99	1.09
14.4.....	147.7	31.0	5.09	1.58
28.8.....	172.6	35.0	4.77	1.67

"size effect" was observed in an analysis of sections at different distances from the tips of branches of plants grown under similar field conditions. The data showed that changes in total area, from the very young stems of approximately 7 mm.² in cross-sectional area to older stems of 90 mm.², were associated with a decrease in bark percentage from approximately 65% to 56% of the total cross-sectional area of the stems. Further increase in stem area, up to 200 mm.², showed only a slight decrease of 1% or 2% in average percentage bark area. The branch areas of the plants supplied with different amounts of nitrogen fall within the sharper portion of this curve. The significant differences in percentage area of bark observed in connection with the nutrient treatments are therefore considered to be due mainly to "size effect." They do not appear to be correlated with rubber concentration of the stems (tables 1, 2).

RESPONSE TO SEASONAL VARIATIONS IN CALCIUM AND NITROGEN SUPPLY.—Very marked increase in vegetative growth resulted when the calcium and nitrogen supply of the plants was increased from 1.8 to 21.5 me. per liter of nutrient (fig. 2; table 3). Greatest growth resulted when plants were supplied with a relatively large amount of calcium nitrate (21.5 me. per liter) during the entire growing season. The percentage of rubber in the stems and roots of plants grown with extremely small amounts of calcium and nitrogen (1.8 me. per liter) was significantly less than that of other plants supplied with larger amounts (7.2–21.5 me. per liter). The over-all production of rubber closely paralleled

TABLE 2

EFFECT OF VARYING THE AMOUNT OF NITRATE ION IN THE NUTRIENT ON RELATIVE PROPORTIONS OF STEM TISSUES, BASED ON COMPARABLE TRANSVERSE SECTIONS FROM BASAL PORTIONS OF REPRESENTATIVE BRANCHES. EIGHT MONTHS AFTER TRANSPLANTING

NITROGEN PER LITER OF NUTRIENT (ME.)	AVERAGE TOTAL STEM AREA (MM. ²)	PERCENTAGE		
		Wood per total stem area	Bark per total stem area	Bark fiber per total bark area
1.4.....	11.1	33.8	66.2	14.5
3.6.....	13.6	31.6	68.4	13.7
7.2.....	23.0	36.2	63.8	13.5
14.4.....	31.8	41.0	59.0	17.3
28.8.....	27.8	40.0	60.0	19.9

the amount of vegetative growth produced by the plants.

The greatest number and best quality of seeds with respect to size and percentage germination were produced by plants that received a relatively large and continuous supply of calcium and nitrogen during the growing season (table 4). Seeds from plants supplied with a rela-

tively small amount of calcium and nitrogen (1.8 me. per liter) were of very low quality, especially those which ripened during July and August, when

fall, while those collected during the warmest part of the growing season were of low quality, irrespective of the nutrient treatment.

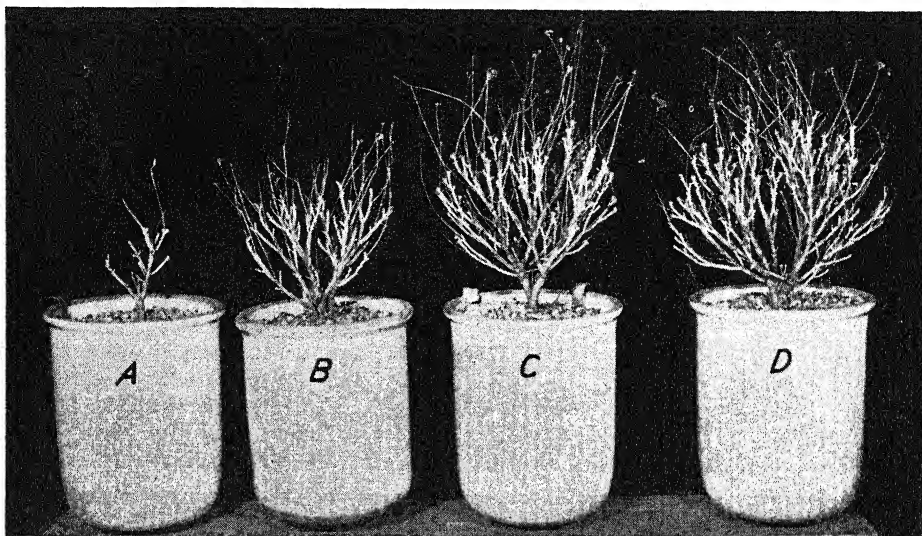


FIG. 2.—Guayule plants grown for 10 months in nutrient cultures containing calcium nitrate: A, 1.8 milliequivalents; B, 7.2 me.; C, 14.4 me.; D, 21.5 me. Leaves removed to show size and extent of branching.

TABLE 3

EFFECT OF VARYING THE AMOUNT OF CALCIUM NITRATE IN THE NUTRIENT ON GROWTH AND AMOUNT OF RUBBER PRODUCED BY GUAYULE. NINE MONTHS AFTER TRANSPLANTING. CHANGES IN COMPOSITION OF NUTRIENT IN TREATMENTS 5, 6, 7, AND 8 MADE IN JULY, APPROXIMATELY MIDDLE OF GROWING SEASON

Treatment no.	Ca(NO ₃) ₂ per liter of nutr ent (me.)	Average fresh weight per plant (gm.)	Average dry weight of stems and roots (gm.)	Average percentage rubber in stems and roots	Average rubber per plant (gm.)
1.....	1.8	46	16.0	3.22	0.52
2.....	7.2	141	43.8	4.51	1.54
3.....	14.4	212	59.0	4.22	2.49
4.....	21.5	269	77.8	4.71	3.66
5.....	1.8 to 21.5	199	48.0	4.61	2.21
6.....	7.2 to 14.4	194	56.4	4.80	2.71
7.....	14.4 to 7.2	167	48.3	4.49	2.17
8.....	21.5 to 1.8	122	38.0	4.63	1.76

relatively high temperatures prevailed. In general, seeds of relatively good quality were obtained during the spring and

Increasing amounts of calcium and nitrogen in the nutrient were associated with great and significant increase in the

total stem and branch areas (table 5). The percentage area of bark of the stem decreased to the extent associated with the "size effect," or changes in the actual area. The increase in the area of wood

nitrogen (1.8 me. per liter) was significantly greater than in plants supplied with a greater amount (21.5 me. per liter). The increase in bark area was somewhat greater than could be ac-

TABLE 4

EFFECT OF VARYING THE AMOUNT OF CALCIUM NITRATE IN THE NUTRIENT ON PERCENTAGE GERMINATION OF SEEDS PRODUCED BY GUAYULE. CHANGES IN COMPOSITION OF NUTRIENT IN TREATMENTS 5, 6, 7, AND 8 MADE IN JULY, APPROXIMATELY MIDDLE OF GROWING SEASON

TREATMENT NO.	Ca(NO ₃) ₂ PER LITER OF NUTRIENT (ME.)	PERCENTAGE GERMINATION			
		June 1 to July 15	July 15 to Aug. 9	Aug. 9 to Sept. 1	Sept. 1 to Oct. 16
1.....	1.8	17.5	1.5	6.0	20.0
2.....	7.2	20.3	8.0	26.5	27.5
3.....	14.4	15.0	7.3	37.5	29.8
4.....	21.5	15.3	6.8	31.7	36.3
5.....	1.8 to 21.5	19.2	12.3	22.0	26.8
6.....	7.2 to 14.4	16.8	5.8	29.8	29.5
7.....	14.4 to 7.2	12.5	6.3	44.5	25.3
8.....	21.5 to 1.8	12.8	8.5	29.5	22.5

TABLE 5

EFFECT OF VARYING THE AMOUNT OF CALCIUM NITRATE IN NUTRIENT ON RELATIVE PROPORTIONS OF STEM TISSUES, BASED ON COMPARABLE TRANSVERSE SECTIONS FROM BASAL PROPORTIONS OF REPRESENTATIVE BRANCHES. NINE MONTHS AFTER TRANSPLANTING. CHANGES IN NITROGEN CONCENTRATION OF NUTRIENT IN TREATMENTS 4 AND 5 MADE IN JULY, APPROXIMATELY MIDDLE OF GROWING SEASON

TREATMENT NO.	Ca(NO ₃) ₂ PER LITER OF NUTRIENT (ME.)	AVERAGE TOTAL STEM AREA (MM. ²)	PERCENTAGE		
			Wood per total stem area	Bark per total stem area	Bark fiber per total bark area
1.....	1.8	12.2	31.2	68.8	24.5
4.....	21.5	51.2	42.6	57.4	27.0
5.....	1.8 to 21.5	40.1	39.3	60.7	29.5
8.....	21.5 to 1.8	34.6	35.6	64.4	29.1

was correlated with the decrease in the percentage area of bark. There were no significant changes in the fiber in the bark of the stem. In the branches, the percentage area of the bark in plants grown with low amounts of calcium and

counted for by the change associated with size.

Discussion

In previous experiments, the vegetative growth of guayule plants was con-

trolled by altering their external supply of boron (5) and by subjecting them to various light intensities (6), with the result that decreased vegetative growth during the summer growing season was associated with a decrease in the concentration of rubber in the plant as measured at the end of the following winter dormancy period. In the present experiments, the vegetative growth of guayule was controlled to some extent by controlling the nitrogen, or calcium and nitrogen, supply. Some plants were made to grow vigorously during the entire growing season, while others grew vigorously during the first half of the season but more slowly during the latter part; still others were made to grow slowly at first and then rapidly during the latter part of the summer growing season.

Except for extreme nitrogen and calcium starvation, the percentage of rubber in stems and roots of plants supplied with limited amounts of these elements, or of nitrogen alone, was not significantly different from that of plants grown with an optimum supply. Plants grown with a nutrient deficient in nitrogen showed relatively small growth during the summer, and at the lower nitrogen levels showed extreme symptoms of nitrogen deficiency in the fall, but they attained a rubber concentration during a winter dormancy period equal to that of others which had grown vigorously during the entire summer as the result of adequate nutrient supply. Checking the vegetative growth of plants during either the first or the latter part of the growing season by decreasing the external nitrogen and calcium supply did influence the accumulation during the following winter dormancy period, as indicated by the concentration of rubber in stems and roots. In these experiments, nutrient treatments favorable to maximum growth

were likewise favorable to high rubber yields, mainly because of the relatively large size attained by the plants supplied with adequate nutrient, rather than because of the ability of such plants to synthesize more rubber per unit of plant weight.

Since rubber is stored mainly in the bark of guayule, attention has been directed toward its thickness in plants subjected to various environmental conditions. The relative over-all area of the bark and the area of the bark occupied by non-rubber-bearing fiber have been used, with proper consideration of "size effect," as an index to the volume of rubber-storing tissue per unit length of stem. In the present experiments, the diameter of the stems varied in proportion to the amount of nitrogen supplied to the plants. The relative area of bark was essentially constant, except in those treatments supplying very small amounts of nitrogen and calcium, and possibly of nitrogen alone. In previous experiments (5), plants supplied with varying amounts of boron showed marked differences in stem diameters but possessed proportionately constant percentage areas of bark, except in the case of those plants grown with a deficiency of boron. Variation in the intensity of light to which the plants were exposed has also a marked effect on stem diameter but was ineffective in changing the relative proportion of bark and wood (6). LLOYD (4) and ARTSCHWAGER (2) report that the bark in nonirrigated plants was relatively thicker than in irrigated plants. ADDICOTT and PANKHURST (1) found that low—as compared with high—moisture stress increased cambial activity, which resulted in greater areas of both xylem and phloem; and with intermediate stress the development of xylem appeared to have priority over that of

phloem. In general, the results of our anatomical studies indicate that the relative proportions of bark and wood in the stems of guayule plants remain essentially constant, irrespective of relatively wide variations in the nutrient supply—except that conditions of deficiency may result in a slightly increased proportion of bark. ADDICOTT and PANKHURST also concluded that it is apparently difficult to modify the fundamental pattern of development in guayule by the physiological condition of the plant. This general constancy of proportion is not correlated with the total rubber output of the plant, since the latter may be greatly influenced by variation in nutrient supply.

Summary

1. The growth of guayule in gravel cultures was controlled by supplying the plants with nutrient containing various concentrations of calcium and nitrogen ions. Some plants were made to grow vigorously during the entire summer growing season, the growth of others was checked during the entire season, others were made to grow vigorously during the first half of the season and then slowly, while still others grew slowly during the first part of the season and then vigorously during the latter part of the summer. All were subjected to the same light and temperature conditions during the winter dormancy period.

2. The amount of vegetative growth made during the summer season was not related to the plant's ability to synthesize rubber, as based on the percentage

found in the stems and roots following the winter dormancy period. Small plants showing nitrogen deficiency symptoms were as efficient with respect to rubber storage as were relatively large plants grown with a much greater nitrogen supply. The total rubber production was directly related to the amount of growth made by the plants during the summer growing season.

3. Seeds of low quality with respect to percentage germination were produced during the warmest period of summer, irrespective of the amount of calcium or nitrogen supplied to the plants. Seeds collected during the spring and fall gave a relatively high percentage germination, and their number and quality were favored by adequate nutrient supply.

4. The relative area of bark of plants grown with varying but not deficient amounts of nitrogen was essentially constant when the inherent differences due to variation in the size of the stem were discounted. A slight increase occurred in the relative thickness of the bark in branches of plants supplied with relatively small amounts of calcium and nitrogen. This increase was not associated with an increase in the percentage rubber in the stems.

The rubber determinations were made by R. L. HOLMES and the statistical analyses by MARY W. SHANOR, of the Special Guayule Project.

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VARIETIES OF KENAF (*HIBISCUS CANNABINUS*), A BAST FIBER PLANT, IN CUBA

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Introduction

In 1942, seeds from plants of *Hibiscus* were introduced into Cuba from El Salvador under the name of roselle (*Hibiscus sabdariffa* var. *altissima*), in an effort to determine the possibilities of producing this plant as a source of soft fiber for the manufacture of burlap and other articles in which jute (*Corchorus capsularis*) fiber is used. This plant offered a speedy source of material, as the soft fiber obtained from the bast layer or the tissue just under the bark of the stem is ready for harvesting about 90 days after the crop has been planted. Recently, however, the identity of the plant has been established as kenaf (*Hibiscus cannabinus* L.), which—under favorable soil and climatic conditions—is capable of producing more than a ton of dry fiber per acre.

The seed introduced from El Salvador was a heterogeneous mixture containing at least two varieties of this species, as evidenced by the fact that all the plantings in Cuba observed by the writers have been found to contain two morphological types of plants—one having

digitate leaves and the other having cordate ones. The majority of the plantings contained about 75% of the digitate type, but in a few instances the proportion was about half digitate to half cordate. The plants grew to an equal height and were alike with respect to growth habit, color of leaves, stems and flowers. But one variety was characterized by having cordate leaves at the bottom of the plant; 3-, 5-, and 7-palmately lobed leaves, respectively, up the middle; and 3-palmately lobed leaves on the top portion. On the other hand, the second variety was characterized by having only cordate leaves throughout the entire length of the stem. This situation was perplexing to local producers as well as to research workers investigating the plant in Latin America.

Investigation

SPECIES AND VARIETIES

Hibiscus sabdariffa may be distinguished from *H. cannabinus*, according to RAO and SESHADRI (8), by differences in color of the flower petals after they have been picked from the plant. The petals of *H. sabdariffa*, which are pale yellow in color, acquire a red tinge after removal, while those of *H. cannabinus* do not.

According to McVAUGH (6), these

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species also exhibit the following differences in seed characteristics (fig. 1):

H. cannabinus.—"Seeds generally triangular, angles rather acute; surface dull gray with numerous conspicuous yellowish brown raised spots; hilum yellowish brown, relatively small.

H. sabdariffa.—"Seeds generally rounded-hemispherical, angles rounded; surface dull gray

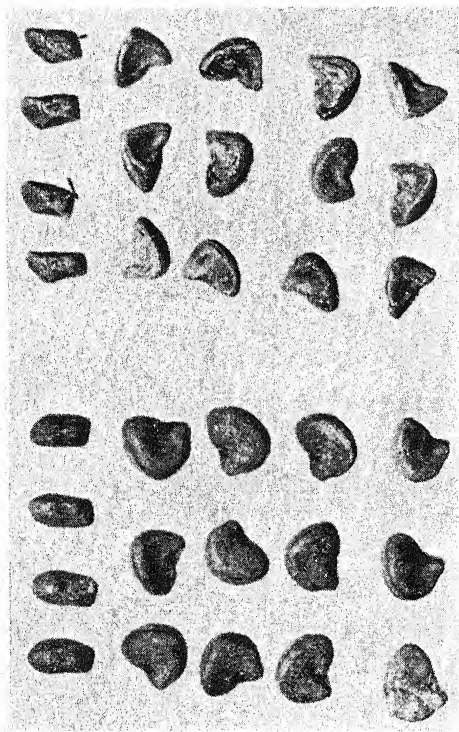


FIG. 1.—Seeds of *Hibiscus*. $\times 2$: Those of *H. cannabinus* (top) are generally triangular in shape, with acute angles; those of *H. sabdariffa* (bottom) are generally rounded-hemispherical, with rounded angles. Photographs of this and of succeeding illustrations by JAMES T. MITCHELL, Office of Foreign Agricultural Relations, U.S.D.A.

without spots; hilum brownish red, relatively large."

In addition to these differences in color of withered flower petals and seed characters, there are also noteworthy differences in various floral parts and in

the appearance of their stems, as evidenced by the following key and botanical descriptions of HOOKER (3):

"Bracteoles more than 5, free or connate at the base, not adnate to the calyx.

H. cannabinus L.; DC. Prodr. i. 450; annual or perennial, prickly, stem glabrous, lower leaves entire upper lobed, mid-nerve glandular beneath, peduncle very short, bracteoles 7-10 linear, shorter than the calyx, sepals glandular. . . .

Stem glabrous, prickly. Lower leaves cordate, upper deeply palmately lobed, lobes narrow, serrate; petiole prickly, lower much longer than the blade. Stipules linear, pointed. Peduncles axillary, very short. Sepals bristly, lanceolate, connate below the middle, with a gland at the back of each. Corolla large, spreading, yellow with a crimson centre. Capsule globose, pointed, bristly. Seeds nearly glabrous. . . .

Bracteoles adnate to the base of the calyx.

H. sabdariffa L.; DC. Prodr. i. 453; annual, glabrous, unarmed, stem purplish, leaves entire or lobed, glandular beneath, peduncles very short, thickened at the summit, bracteoles 8-12 linear, adnate to the base of the calyx, sepals bristly. . . .

Erect. Leaves polymorphous, midrib glandular beneath; petiole 2 in. Peduncle and calyx accrescent. Sepals deltoid, acuminate, connate below the middle into a purplish fleshy cup. Corolla $2\frac{1}{2}$ in. diam., yellow. Capsule ovoid, pointed, villous, shorter than the calyx. Seeds reniform, sub-glabrous. . . ."

The differences included in the preceding descriptions are summarized in table 1. According to these identifying characters, the plant material in Cuba evidently corresponds with *Hibiscus cannabinus* and not with *H. sabdariffa*.

Perhaps the most comprehensive botanical investigation conducted on the species *cannabinus* was by HOWARD and HOWARD (5) in India. From the material they collected, the following five varieties comprising eight agricultural types were selected and described:

"1. Variety *simplex*

Type 1.—Stems purple; leaves entire with purple petioles.

2. Variety *viridis*
Type 2.—Stems green; leaves entire with green petioles.
3. Variety *ruber*
Type 3.—Stems red below, greenish above; leaves divided with green petioles.
4. Variety *purpureus*
Stems purple; leaves divided with purple petioles.
Type 4.—Late, stems very tall and slender; leaves with narrow lobes of a diffused purple colour; petals purplish.
Type 5.—Early; stems short and robust; leaves green with broad lobes.

base numerous stout branches which grew parallel to the main stem. They recommended the three types of variety *vulgaris* with their long unbranched stems for fiber production. Investigations in Cuba have shown that there is little difference in the ultimate height of these two varieties and that their tendency for branching is practically identical. Variety *viridis*, planted at four different planting distances, branched only 0.4% more than did *vulgaris*, planted at

TABLE 1
COMPARATIVE DIFFERENCES BETWEEN *HIBISCUS CANNABINUS* AND *H. SABDARIFFA*

Plant part	<i>H. cannabinus</i>	<i>H. sabdariffa</i>
Flower:		
Bracteoles.....	Not adnate to calyx	Adnate to base of calyx
Color of corolla		
Fresh.....	Pale yellow to sulphur	Pale yellow
Withered.....	Pale yellow to sulphur	Pale yellow with reddish tinge
Seed:		
Shape.....	Triangular, angles acute	Rounded-hemispherical, angles rounded
Surface.....	Dull gray with many yellowish brown, raised spots	Dull gray without spots
Hilum.....	Yellowish brown, relatively small	Brownish red, relatively large
Stem.....	Prickly	Unarmed

5. Variety *vulgaris*
Stems green; leaves divided with green petioles.
Type 6.—Plants very early.
Type 7.—Plants late; seedlings with reddish stems.
Type 8.—Plants late; seedlings with green stems."

By comparing the color plates and the descriptions of the material presented by HOWARD and HOWARD (5) with the plant material from Cuba, the writers have been able to identify two varieties of kenaf: *viridis* (type 2) and *vulgaris* (type 8). HOWARD and HOWARD found that variety *viridis* was dwarf in form as compared with the others, and that it had a strong tendency to produce from the

the same distances. Likewise, the percentage fiber content was found to be practically the same in the two varieties.

HORST (4), in Java, obtained seed of seven of the types selected by HOWARD and HOWARD and studied the plants grown from this seed in an effort to determine which of these types were grown in Java and the extent of their adaptability to different locations in that country. From his investigation he strongly recommended varieties *viridis* and *vulgaris*. He stated that *viridis* was grown in the eastern part of Sumatra, whereas in western Java the *purpureus* variety (the "red-stemmed" type) was popular. The green variety *vulgaris*, according to

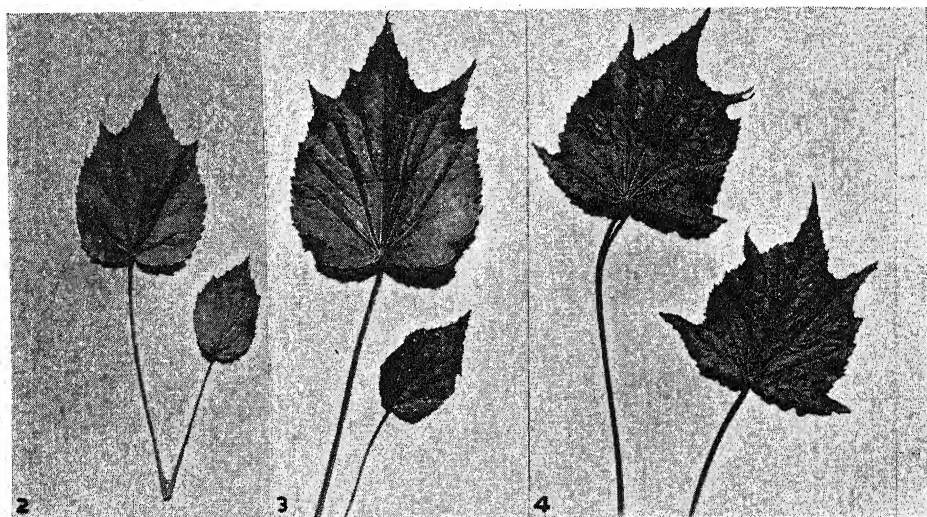
HORST, was the most popular one grown in western Java after the variety *purpureus*. As the seed which was introduced into Cuba from El Salvador was reported to have come originally from commercial plantings in Java, it is logical to assume that the varieties in Cuba are representatives of those grown in Java. Two of these, *viridis* and *vulgaris*, have been identified; the other, *purpureus*, has not been ascertained.

The following description of the two

respectively, up middle portion and 3-palmately lobed into narrow serrate parts at top of stem (figs. 5-7); mid-vein with one gland beneath near base of blade; petioles generally longer than the blades, with prickles slanting toward the blade; stipules linear and pointed.

—*Hibiscus cannabinus* var. *vulgaris* (type 8).

FLOWERS.—Solitary, with short peduncles in axils of leaves; corolla large, spreading, thickened below and thin above, petals pale yellow to sulphur-colored with crimson to purplish center; epicalyx stiff, consisting of 7-8 bracteoles, 10-13 mm. long, which are free above, connate below, and inserted



FIGS. 2-4.—*Hibiscus cannabinus* var. *viridis*: Fig. 2, primary with axillary leaf from bottom portion of stem. Fig. 3, same from middle portion. Fig. 4, leaves from adjacent nodes on top portion of plant.

varieties in Cuba is presented for a basis of comparison:

PLANT.—Herbaceous annual of 3-7 months' duration, depending on time of planting.

STEM.—Straight, simple, more or less glabrous but with prickles; 3-14 feet in height, depending on time of planting.

LEAVES.—Cordate and very shallowly lobed with serrated margins (figs. 2-4), mid-vein with one gland beneath near base of blade, petioles generally longer than the blades, with prickles slanting toward the blade; stipules linear and pointed.—*Hibiscus cannabinus* var. *viridis* (type 2).

Basal leaves cordate and not lobed, 3-, 5-, and 7-palmately lobed into narrow serrate parts,

near base of calyx; calyx bristly, lanceolate, 29-31 mm. long, connate below the middle, 5-parted with one large gland near the middle; style rises through staminal column, one in number which terminates in 5 stigmatic branches; carpels 5, joined into 5-locular capsule, each locule containing 4 to 5 seeds; capsule bristly, globose, pointed; seeds glabrous.

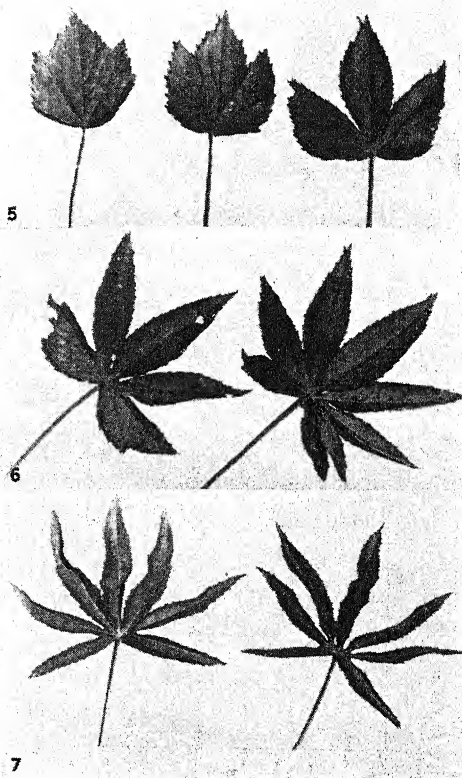
The preceding descriptions indicate that these two varieties are alike in all external characteristics except shape of leaves. USTINOVA (10), however, investigating the percentage of natural cross-pollination in the different varieties,

found that *viridis* was entirely self-pollinated, while in *vulgaris* cross-pollination varied from 2.58 to 39.2% in its different strains. In a previous study (9) she found that irregularities in the structure in the flowers of *vulgaris* were frequent and variable. Although the percentage of staminate and pistillate flowers was not large, nevertheless they did exist. The percentage of undeveloped ovaries in this species was found to vary between 5.5 and 9.5%.

Observations on the two varieties in Cuba have indicated that they are composed possibly of a number of strains, in that there has been some variation in the period of vegetative development among plants. For example, in one particular planting there were a few outstanding plants which were green and had retained all their leaves, while the remainder had dropped most of theirs and seeds had matured. Similar observations have been made by BERLAND (1) in Russia, where he found that *vulgaris* consisted of a number of strains differing by their length of growth period, by their habit of growth and branching, and by some other less important peculiarities connected closely with length of the vegetative period. Strains of the variety *vulgaris* were placed by BERLAND into four groups. The vegetative period of the first group ranged 90-110 days; that of the second, 110-120; the third, 120-130; and the fourth, 130-150 days.

POPOVA (7) showed that the differences in strains of *Hibiscus cannabinus* were in the height of the plant, the thickness of the stem, the color, and in the leaves and flowers. He found strains with large and small seed capsules, and with large and small seeds. He noted that the strains from Central Asia were most frequently mid-season ones, with large capsules and large seeds; whereas the

bulk of strains from Persia (representatives of the variety *vulgaris*) were small-capsuled and small-seeded, with a later maturity than that of the strains from Central Asia. POPOVA further noted that the earlier the strain, the closer to the ground the first flowering node was lo-



FIGS. 5-7.—*Hibiscus cannabinus* var. *vulgaris*: Fig. 5, leaves from adjacent nodes of bottom portion of plant. Fig. 6, 5- and 7-palmately lobed leaves from middle portion of stem. Fig. 7, 7-palmately lobed leaves from adjacent nodes near top portion of stem.

cated; in the late strains, however, flowering began on the upper nodes.

The variations in strains within a particular variety might provide means of improving the plant for fiber purposes by selection in the field for one or two of

the most obviously worthwhile characters, such as height of the first capsule and length of the stem. Some of the characters which an ideal fiber plant should possess are long thin stems, no branches, rapidity of growth, and high fiber percentage with uniform length and strength. As this species is naturally self-pollinated, progress in breeding should be rapid and easy.

POLYMORPHISM OF LEAVES

The accompanying illustrations show pronounced polymorphism of leaves in this species, not only among leaves of different varieties but also among those within the same variety (figs. 3, 5). Cook (2) noted that these variations in leaf forms showed a curious parallel with cotton, a relative of kenaf. He observed that in addition to the entire or very broadly lobed leaves of *viridis* (fig. 2), comparable with those of ordinary Upland varieties of cotton, there were also varieties of kenaf with deeply divided, narrow-lobed leaves, like the so-called "okra" varieties of cotton. Other varieties of kenaf have their leaves parted to the base into narrow digitate segments, a condition also known in some of the tropical varieties of cotton. Further similarity was found, according to Cook, in the fact that *vulgaris* with lobed leaves produced entire leaves at the base of the stalk (fig. 5), as also occurred with the narrow-lobed okra varieties of Upland cotton.

The leaves of the middle and upper part of the stem in *vulgaris* are all deeply lobed (figs. 6, 7), while those of the lower part are without lobes. The transition from one type to the other sometimes occurs between successive nodes (fig. 5), while in other cases this change occupies three or four nodes. A premonition of the change may be found in the larger marginal teeth of the last of the undivided

leaves. A more definitely intermediate condition appears when a leaf is divided on one side but only partially so on the other (center leaf in fig. 5). In cases like this there is a marked difference between the two sides of the leaf, so that the change from the entire to the lobed condition is still quite rapid in comparison with the very gradual changes shown in many plants in changing from the large basal or radical leaves to those of the upper part of the stem.

Cook (2) stated that it was difficult to imagine any practical advantage to be gained by the plants in changing the form of the leaves up the stalk, but he advanced the suggestion that different forms possibly might be connected with the fact that there is a difference of function among the internodes of the stalk. As change of leaf form marks the approach of the fruiting condition in such plants as *Hedera helix* and *Ficus repens*, he thought that the divided leaves in kenaf might indicate in advance the internodes that were to produce flowers and fruit.

The writers have not observed this to be true, however, in that flowering in this plant is dependent on photoperiod; and if planted during the season of the year when the days are short, the plants form flower buds before any divided leaves are evident. As this plant is sensitive to photoperiod, the nodes at which flowering takes place are entirely dependent on time of planting and not on change in leaf form. Although the writers have not made a study of this phenomenon, they are of the opinion that this change in leaf form is perhaps the result of some ecological adaptation.

Summary

1. The species of *Hibiscus* being grown in Cuba for the production of soft fiber has been correctly identified as

kenaf (*H. cannabinus*) rather than as roselle (*H. sabdariffa*). Differences in color of withered flower petals and seed characters, as well as unlikeness in various floral parts and in the appearance of stems, are suggested means of distinguishing these closely allied species.

2. Two varieties of kenaf, *viridis* and *vulgaris*, have been identified as comprising the plant material being grown in Cuba. The botanical descriptions presented show that the two varieties are alike in all respects in morphological appearance, with the exception of their leaves—*viridis* being characterized by having cordate leaves only, while *vulgaris* possesses both cordate and palmately lobed leaves. Differences in plant behavior within a particular variety sug-

gest that these varieties are made up of several strains. These strains might offer a means of improving the plant for fiber purposes by selecting for one or two of the most desirable characters.

3. It is suggested that the differences in leaf shape are perhaps due to some ecological adaptation made during the evolution of the plant.

The collaborative investigations reported here were made possible by funds provided through the United States Interdepartmental Committee on Cultural and Scientific Co-operation, together with the financial support of the Government of Cuba.

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U.S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

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CURRENT LITERATURE

John Torrey. By ANDREW DENNY RODGERS III. Princeton, New Jersey: Princeton University Press, 1942. Pp. 356. Illustrated. \$3.75.

American Botany: 1873-1892. By ANDREW DENNY RODGERS III. Princeton, New Jersey: Princeton University Press, 1944. Pp. 350. Illustrated. \$3.75.

These books, taken together, extend over approximately a century of development of botanical

science in the United States, and for fuller understanding should be considered together. The one covers the life and work of JOHN TORREY. In it are developed details concerning TORREY's teaching, collections, travels, contributions, personal contacts and associations in founding systematic botany, and the establishment of two great herbaria and societies for scientific accomplishment in the United States. Glimpses are given of many of TORREY's contemporaries. Other than TORREY, the individual discussed

at greatest length and in detail is ASA GRAY, who collaborated extensively with him.

The second book, *American Botany*, takes up where the volume on JOHN TORREY leaves off. Now GRAY is made the central figure. In addition to his activities and contributions, those of HOOKER, ENGELMANN, PARRY, LESQUEREUX, GREENE, BRITTON, and many others are discussed, together with sketches on some of the younger men whose work extended for many years after the period covered by this book—1873-1892.

The results of many and varied exploring and collecting expeditions are given.

The author points out that ASA GRAY contributed much in addition to his work as a systematist. His outlook was philosophical, and he gave all scientific enquiry, particularly in the plant field, new impetus. He defended much of the work of CHARLES DARWIN and was conversant with the work of European scholars, both in botany and in horticulture. He was in no sense averse to the enlarging domain of botany into the areas which have become plant pathology, plant physiology, and the whole field of agricultural botany and scientific agriculture. In fact, he directly aided them. As a great teacher he prepared the way for advance through the training of students who took part in the newly and rapidly developing colleges of agriculture and the national experiment station movement.

The author tells something of the institutions concerned with the beginnings of this movement and the lives and work of some of the individuals active in it: ARTHUR, BAILEY, BEAL, BESSEY, BURRELL, HILGARD, and others. The founding of herbaria in addition to those of TORREY and of the United States National Herbarium, several botanic gardens, and several scientific organizations are noted. In fact, the number of individuals concerned with botanical investigations, the kinds and numbers of problems undertaken, and the places at which such work was carried out show so great and rapid increase during the decades discussed that the reader becomes all but lost in his attempts to follow them. Even so, a careful reading of this and the preceding volume affords rich reward to anyone who desires a reasonably clear background for an understanding of the evolution of the even more widespread work in the theoretical and applied botanical problems of the present.—E. J. KRAUS.

Root Disease Fungi: Vol. I. By S. D. GARRETT. Waltham, Mass.: Chronica Botanica Co.; New York City: G. E. Stechert & Co., 1944. Pp. 177. \$4.50.

The appearance of this book marks the first volume of *Annales Cryptogamici et Phytopathologici*, edited by Frans Verdoorn. The author stresses the

relationships between the inciting agents of these diseases and their habitats—the soil. The book is an interesting ecological study of an important group of causal agents within the larger field of root diseases of plants. The author has limited the scope of the presentation even more by not dealing “comprehensively either with all root-infecting fungi, or with the disease that they cause.” Nevertheless, the book is a useful contribution to microbiological and phytoparasitological literature. The author concludes that the soil environment of microorganisms and plants varies less widely than the above-ground environment, especially in cultivated soils; that the problems of soil-disease control essentially are the same in tropical crops and in crops of the temperate regions; and that methods of control vary less with the region than with the type of cultivation, namely—“field, plantation, and glass-house cropping.”

The first three chapters and chapters 7 and 8 are devoted to a consideration of the parasitism and saprophytism of root-infecting fungi. Chapters 8-15 concern themselves with control of root diseases incited by fungi.

The volume includes a bibliography, a general index, and an author's index. It is well written and the illustrations are excellent.—G. K. K. LINK.

A Manual of Soil Fungi. By JOSEPH C. GILMAN. Ames, Iowa: Collegiate Press, Inc., 1945. Pp. 392. Figs. 135. \$5.00.

The purpose of this volume is to provide a tool in the identification of soil fungi. It includes chiefly those species which have been cultivated artificially on various types of biological media, excluding the terrestrial mushrooms; the plant pathogens (which though considered soil-borne have not been isolated directly from the soil); and the forms reported from leaf mold, decayed wood, or other substances not yet fully incorporated in the complex known as soil.

A brief introduction precedes the main body of the book, which consists of keys and species descriptions. The general keys are based on LINDAU's System in ENGLER and PRANTL's *Die natürlichen Pflanzenfamilien*. Species keys and descriptions are garnered from various pertinent monographs. More than half the pages are devoted to the Fungi Imperfecti, and all of these—save four—to the Moniliales. Of these 196 pages, 57 are taken up by 124 species of *Penicillium*, and 38 pages by 428 species and 24 varieties of *Fusarium*. The Saprolegniaceae occupy 61 pages, with the genus *Achlya* dominant with 25 species; the Mucorales require 47 pages, with the genus *Mucor* dominant with 42 species.

A bibliography, a glossary, and an index conclude the volume, which should prove useful to those interested in soil fungi.—G. K. K. LINK.

CYTOLOGY AND BREEDING BEHAVIOR OF SELECTED
PLANTS OF *POA PRATENSIS*¹ETTLAR L. NIELSEN²

Introduction

Poa pratensis L. is one of the most ubiquitous perennial grasses occurring in the cooler humid portions of North America. It is also common throughout northern Europe. Its widespread occurrence as a forage and turf species in these regions has provoked considerable interest in the development of new types. MÜNTZING (26) demonstrated that an apomictic form of reproduction occurred in this species. Its economic importance and its peculiarities in reproduction have resulted in an extensive bibliography.

Examination of the literature concerning the cytology and breeding behavior of the bluegrasses reveals that much of the information is fragmentary and inconclusive. This may be partially attributed to the fact that such researches represent for the most part studies of isolated portions of the complete life history. It appeared desirable, therefore, that the scope of the present study be broadened and that certain critical phases of the entire life cycle of the plant be examined in some detail. Although such an investigation was undertaken, numerous gaps in the data still remain. A number of these, such as the compara-

tive developmental embryology in sexual and apomictic plants, are apparent.

Material, methods, and terminology

Four families of related plants were selected for this study. The original seed lot of *P. pratensis*, from which the progenies were developed, was received from W. H. Wright of the Dominion Department of Agriculture, Ottawa, Canada. Seedlings were established in the field in 1936. Another progeny was established in 1938 from a single panicle of a plant selected in 1937. Four plants were selected from this progeny in 1939. One of these was considered as typical of the predominant morphological type of the family and three were representative aberrant plants. Seedlings from individual panicles harvested from these four plants were established in the field in 1940.³ In 1941, adult plants of three progenies of the twenty-four plants each exhibited diverse morphological types, whereas only two aberrant plants were in evidence in the fourth.

These four progenies are described as follows. The types indicated refer to groups of plants of similar growth and morphological characteristics. They may or may not be identical plants such as would be expected from apomictic seed formation.

70. Developed from an aberrant plant. 91.7% of the progeny were aberrant and of varying morphological types. Two plants (type

¹ Results of co-operative studies between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering and the Wisconsin Agricultural Experiment Station, Madison.

² Associate Agronomist, Division Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U.S. Department of Agriculture.

³ Progenies from 1936 to 1940, inclusive, were grown by Drs. F. W. Tinney, O. S. Aamodt, and H. L. Ahlgren.

I) were morphologically similar to the predominant type of No. 71. Eleven plants (type II) approached the preceding in morphology but were less vigorous. Three plants (type III) were of tussock-growth habit, medium-coarse textured, and moderately leafy. Eight plants (type VII) were of varying gross morphology, no two of which appeared similar.

71. Developed from a parental-type plant (type I). These were vigorous, moderately strong creepers, leafy, and of medium texture. Two plants (8.3%) of the progeny were aberrants (type VII). Both were of semitussock-growth habit with numerous long leaves.
72. Developed from an aberrant plant. 95.8% of the progeny were aberrants. One plant (type I) was similar to the predominant type of No. 71. Three plants (type II) were essentially similar to the preceding but less vigorous. Twenty plants (type VII) were of varying morphology, no two being closely similar.
73. Developed from an aberrant plant. 87.5% of the progeny were aberrants. Three plants (type I) were similar to the predominant type of No. 71. Nine dwarf, bunch-type plants (type IV) had dark, fine-textured leaves and purple panicles. Two nonaggressive erect plants (type V) had medium-coarse leaves, and eight (type VI), which were semidwarf, had fine, dark-green leaves and purple panicles. These were somewhat more aggressive in growth habit than those of type IV. There was also one variant plant (type VII) that did not fall into any of these classes.

The data for aberrant frequency given in the description of progenies are based upon similarity to the predominant type of progeny 71. If, however, the aberrant frequency is calculated upon the basis of the predominant type in a given progeny, the aberrant frequencies are 8.3, 58.3, 87.5, and 62.5% for progenies 70, 71, 72, and 73, respectively.

Material for embryological studies was taken at regular intervals from the time of the emergence of the tip of the panicle until caryopses were at the dough stage. This was fixed in Sax's modifica-

tion of Navashin's and/or Allen's modification of Bouin's fluids. These preparations were transferred 24 hours after fixation to 70% alcohol for storage. Later random samples from each fixation were imbedded, sectioned at 12–25 μ (depending upon the age of the floret), and stained in Heidenhain's or/and Delafield's haematoxylin.⁴

Other materials for smear preparations were fixed in acetic alcohol and/or chloroform-acetic alcohol. This was transferred to 70% alcohol 48 hours later, sealed, and stored until used.

During September, 1941, 6-inch cores from each of the selected plants were transplanted into an isolation plot in soil of moderately low fertility adjacent to the site to be used for progeny tests. These were for comparison with the plants of their progenies.

Selected panicles were harvested and placed in individual envelopes for storage. During early March, 1942, all the seeds from a single panicle were planted in small bread pans filled with a friable sandy loam compost. Seedlings taken at random were transplanted to individual plant bands when they were about 2–3 inches tall. These were subsequently transplanted into spaced rows in the field in May.

TERMINOLOGY.—During the interim of years that have passed since WINKLER (42) defined apomixis, an extensive bibliography concerning this phenomenon has been developed. Confusion has arisen in the usage of the terms proposed by different workers for variations in asexual reproduction. In this paper the terminology proposed by FAGERLIND (15) and

⁴ Duplicate sets of herbarium specimens were prepared from each of the plants selected for embryological study. One set was retained at Madison, Wisconsin, and the other deposited in the Herbarium of the Smithsonian Institution in Washington, D.C.

by STEBBINS (39) is followed. The terms in question are:

Apomixis.—“Apomixis is the substitution for sexual reproduction of another, asexual reproductive process that does not involve nuclear or cellular fusion (that is fertilization)” (WINKLER, 42, as given by STEBBINS, 39, p. 509).

Somatic apospory.—“The embryo-sac initial is a purely somatic cell usually of the chalazal region, but sometimes of the nucellus” (39, p. 511).

The terms “parental” and “aberrant” are used in this paper to describe morphological similarity or dissimilarity of progeny members as compared with the parent seed plant. An aberrant differs morphologically from the seed parent. A plant of a progeny that resembles its seed parent is referred to as the parental type.

Results

SEED SET, GERMINATION AND SEEDLINGS

LITERATURE REVIEW.—

ZOLLIKÖFER (43) studied the establishment of plants of *Poa alpina* L. from seeds and from proliferated propagules. Generally, the vigor of plants established from seed was inferior to that of young plants from proliferations. She suggested that the formation of the few seeds in plants of this species might be of importance for the evaluation of the apogamy question in species of *Poa*.

NILSSON (31) observed differences in the “cross- and self-fertility” of biotypes of *P. pratensis*. He reported that no seed formed in emasculated florets unless pollination had been effected subsequently.

ÅKERBERG (1, 2) and MÜNTZING (27) reported on the quantitative variation in the amount of seed produced by biotypes of *Poa* species of varying morphological

characters. In 1942, ÅKERBERG (5) reported differences in the amount and germinability of seeds among plants in progenies of *P. pratensis*.

SPENCER and FERGUS (37) found variations in the percentages of caryopses developed in florets in seed lots of *P. pratensis* harvested in Kentucky and Ohio. Eight types were classified on the basis of floret and ovarian development. At harvest time the most poorly developed florets contained both sterile stamens and undeveloped pistils, both of which probably had been arrested during their early stages. Intermediate stages were classified on the basis of apparently functional anthers, partial development of the ovary, completely developed but shriveled seeds, seeds with opaque endosperm, and mature seeds with hard endosperm. They found no ovarian development in 46% of all the florets of the 1941 seed crop. Seventy-two per cent of the florets had incompletely developed seeds. Of the remaining, only 22.5% had developed seeds with hard endosperm, and 5% were either of soft or of soft and hard endosperm.

EXPERIMENTAL RESULTS.—

In March, 1942, marked differences were found in the germination of the seed from panicles of different plants. Seedling emergence varied from 7 to 12 days after planting, and the number varied from two or three individuals to more than 400. Although the panicles from the different plants varied somewhat in size and in the number of florets, the variation in the number of seedlings that emerged could not be explained entirely on the basis of difference in the size of the panicles. The relative numbers of seedlings produced from a panicle of the different plants are given in table 1.

Irrespective of the progeny, generally

there was no consistent, close relation among plants classified as morphologically similar and the number or vigor of the seedlings derived from a single panicle. For illustrative purposes, plants 70-20, 70-22, 73-11, and 73-21 were classified as morphologically similar (type I), but they differ markedly in the number and vigor of seedlings developed from the seeds of individual panicles.

Seeds from panicles that had been harvested in June, 1943, were planted in September and handled similarly to those grown earlier. The results of this planting very closely paralleled those of the previous year. Of the forty-one seed lots studied, five differed somewhat in the number of seedlings produced. Four of these were parental-type plants (71-6 and 10, and 73-19 and 21). The fifth was an aberrant (71-3).

Because of the lack of exact fundamental information concerning these relative observations pertaining to the seed and seedlings from different plants, duplicate seed lots from other panicles harvested in 1943 were germinated, using regular seed laboratory technique. The data given in table 1 show that the numbers of seeds in a panicle from these selected plants of four sister progenies are highly variable. The lowest average number of seeds, eleven, was produced in a panicle of plant 70-19, and the highest average number, 213, in a panicle of plant 72-20. The isolated plants were somewhat less vigorous in 1943 than in 1941. Panicles therefore produced somewhat fewer seeds in 1943.

The germinability of the seed produced was generally high. Of the forty-four lots tested, thirty-six germinated 80% or higher. Plant 72-19 produced thirty-four seeds, of which only two germinated; and from sixteen seeds of 70-2 only two seedlings developed.

There is fair agreement between the

number of seedlings developed in pans in 1942 and the number of seeds and seedlings developed from the same plants in 1943. Exceptions are evident in table 1, but the statement appears to be generally true.

From these observations concerning the relative number of seedlings produced from the seeds of individual panicles from the several plants, and from the germination studies, it appears that in most cases the number of seedlings produced is determined at some stage in the ontogenetic development of the ovules rather than in the senescence of completely developed seeds.

PROGENY TESTS

LITERATURE REVIEW.—

MÜNTZING (26) found aneuploid chromosome numbers of matroclinic plants of uniform progenies to be similar to those of the maternal plants of *Poa alpina* and *P. pratensis*. RANCKEN (35) confirmed these results for *P. pratensis*. FLOVIK (16) reported similar breeding behavior in *P. alpigena* (E. Fries) Lindm. type *domestica* ($2n = 84$), *P. glauca* Vahl ($2n = 70$), and *P. arctica* R. Br. ($2n = 56$).

ÅKERBERG (4) reported an average of 9.2% of aberrant plants after "isolation, free-flowering and crossing" apomictic biotypes of *P. pratensis*. Collections from wild populations produced progenies with 5.9% aberrants. BROWN (12) found 0.1-18.2% aberrant plants in progenies from seeds produced by open pollination.

MÜNTZING (27) studied F_2 and F_3 progenies of a sexual *P. pratensis* plant and concluded that apomictic seed formation is recessive to sexual propagation. It is also recessive in hybrid, haploid, and triploid plants. The cross sexual \times apomictic, in *P. alpina*, tended to give haploids, indicating apomictic tend-

TABLE 1

NUMBER OF SEEDLINGS AND PERCENTAGE GERMINATION OF SEED FROM INDIVIDUAL PANICLES AND THE SEGREGATION IN PLANT PROGENIES OF *POA PRATENSIS*

TYPE	PLANT	NO. SEEDLINGS FROM SINGLE PANICLE IN			PROGENY BEHAVIOR	
		Pan*	Germinator		No. plants	Aberrant (%)
			No. seeds	Germination (%)		
		Family 70†				
I	70-20.....	2	163	71	21	90.5
	22.....	5	75	79	24	37.5
II	11.....	4	74	96	18	100.0
III	13.....	5	95	82	22	63.6
	17.....	4	89	87	19	63.2
	19.....	5	11	64	24	58.3
VII	2.....	5	16	13	23	39.1
	6.....	2	66	91	24	50.0
	8.....	3	39	41	21	57.1
	18.....	5	88	93	24	25.0
Total and weighted average.....					220	56.8
		Family 71				
I	71- 6.....	2	102	87	24	25.0
	10.....	2	99	97	19	100.0
	12.....	2	48	92	24	16.7
VII	3.....	2	97	97	23	26.1
	8.....	5	82	90	20	35.0
Total and weighted average.....					110	38.2
		Family 72				
I	72- 5.....	4	184	99	19	0.0
II	11.....	5	47	83	23	100.0
	12.....	5	27	100	20	45.0
	19.....	5	34	6	22	72.7
VII	1.....	5(2, 0)†	82	70	2	50.0
	6.....	5	66	91	24	100.0
	7.....	5	19	89	19	100.0
	8.....	2	82	90	23	100.0
	9.....	5(2, 3)†	102	99	4	50.0
	13.....	5(3, 13)†	46	96	5	20.0
	14.....	1	154	97	24	8.3
	16.....	3	44	93	23	100.0
	17.....	2	109	90	23	8.7
	18.....	4	47	85	23	100.0
	20.....	5(14, 18)†	213	97	19	73.7
	22.....	5(14, 4)†	56	89	8	62.5
	23.....	5(8, 180)†	160	93	20	100.0
	24.....	5	32	88	24	100.0
Total and weighted average.....					325	71.1

* Seedling classes: 1, numerous; 2, many; 3, medium; 4, few; 5, very few.

† Segregation of plants of family 70: two type-I plants, eleven type-II plants, three type III, eight type VII. Family 71: twenty-two type I, two type VII. Family 72: one type I, three type II, twenty type VII. Family 73: three type I, nine type IV, two type V, eight type VI, one type VII.

‡ Actual number of seedlings from first and second plantings, respectively.

TABLE 1—*Continued*

TYPE	PLANT	No. SEEDLINGS FROM SINGLE PANICLE IN			PROGENY BEHAVIOR			
		Pan*	Germinator		No. plants	Aberrant (%)		
			No. seeds	Germination (%)				
I IV V VI	73-II..... 19..... 21..... 1..... 4..... 8..... 14..... 2..... 5..... 15..... 16.....	Family 73						
		5	83	95	24	62.5		
		3	92	93	24	29.2		
		2	24	16.7		
		5	27	89	23	69.6		
		5	22	22.7		
		5	24	83	21	47.6		
		5	64	84	18	100.0		
		5	172	94	24	25.0		
		5	43	56	24	70.8		
		5	18	44.4		
		5	14	35.7		
		Total and weighted average.....					236	34.0

encies toward development without fertilization. The absence of apomictic F_3 progenies of *P. pratensis* was considered definite evidence that apomictic seed formation is conditioned by more than one genetic factor. The balance of these factors may be upset by crossing or by quantitative chromosomal changes. Divergence in type within a chromosomal group was considered due to mutation.

ENGELBERT (13) concluded from her breeding experiments that races of *Poa arctica*, *P. alpigena*, *P. alpina*, and *P. pratensis* were "apomictic (pseudogamous)."

TINNEY and AAMODT (41) reported no variant plants in thirty-eight of 102 individual plant progenies of *P. pratensis*. The highest percentage of aberrants reported was 21.9. The total population averaged 1.6% variant plants.

BRITTINGHAM (10) analyzed 115 progenies of the same species. Four were com-

pletely uniform in type. Sixty-two had 0.1-12% aberrant plants. Of the remaining forty-eight progenies, forty-one had 13-43% and eight had 44-65.5% aberrants. There was an average of 14.8% aberrants in the total population.

MYERS (30) selected forty-six plants from three progenies that had been studied previously by BRITTINGHAM, who had found that they contained 3, 27, and 48% variant plants, respectively. From the breeding behavior of these forty-six plants he concluded that "variant type plants produced a higher proportion of sexual seeds than did their parents of parental type sibs."

EXPERIMENTAL RESULTS.—

Forty-four progenies from plants of families 70-73, inclusive, were established in May, 1942. They were examined for vigor and general uniformity in the fall and again in 1943. Characters

used in determining variations from the parental plant type included early vigor, color, growth habit, aggressiveness, texture of foliage, earliness of vegetative growth, early heading date, relative date of ripening, number of culms, coarseness of culms, vigor at maturity, height, panicle type, late growth habit, leafiness, and leaf length.

The data concerning aberrant frequency among the progenies of the selected plants are given in table 1. Certain significant features of these should be mentioned.

Considering first the progenies from plants classified as parental type (I), there is a wide range in percentages of aberrants (figs. 1, 2). The progeny of only one plant (72-5) was uniform. Other percentages of aberrants ranged from 17 to 100%.

The type-II plants were classified on a basis of being similar in morphology to the plants of type I but somewhat less vigorous. No plant placed in type II had more than 65% of its progeny classified as similar, and in two cases totally diverse populations were encountered.

Likewise, in any other group of plants classified as similar morphologically (types III, IV, V, and VI), diverse percentages of aberrants appear in their progenies. Type III of family 70 and type VI of family 73 are the only ones wherein reasonably comparable percentages of aberrants occurred in the progenies of the three plants classified as similar.

Two plants of type VII of family 72 produced progenies with 8-9% aberrants. Hence, two plants that reproduced primarily by apomixis appeared among the twenty morphologically diverse aberrants of the previous generation. Seven of the fourteen different aberrant plants studied produced totally diverse prog-

enies, indicating at least frequent if not entirely sexual reproduction. From this it may be assumed that in some florets of otherwise sexual plants there is a change toward apomictic reproduction. The converse also appears true in some instances (figs. 2-7).

Plants of types IV and V of family 73 were considered as dwarfs or semidwarfs. Some plants appeared in the progenies from these dwarfs and semidwarfs that were much more vigorous and differed morphologically from their parental-type sister plants. An example of these is shown in figure 8.

From these data it appears that the morphological characteristics of plants are not necessarily related to breeding behavior, nor does there appear to be any direct relationship between seedling development and progeny behavior.

Cytology

MACROSPOROGENESIS AND EMBRYOLOGY

LITERATURE REVIEW.—

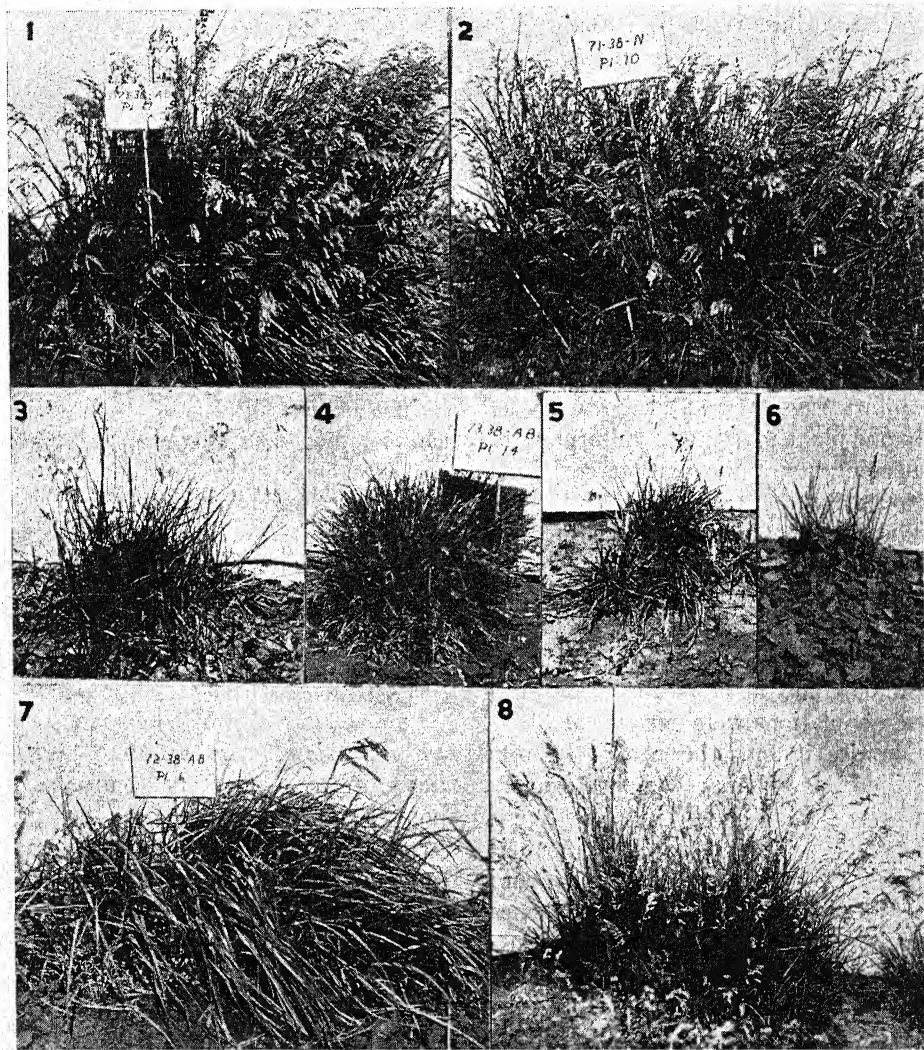
Two reports on the embryology of *Poa pratensis* appeared prior to MÜNTZING's (26) proposal of the occurrence of apomixis in bluegrass. NISHIMURA (32) discussed the floral primordia, the mature sac, and the young embryonic stages. The critical stages of the reduction divisions and the haploid macrospores were not considered.

ANDERSON (7) did not report observing the collapse of the macrospores in *Poa pratensis* and *P. compressa* L. Figure B of her plate 2 might suggest that the binucleate embryo sac was derived from a somatic cell lying adjacent to the macrospores. Multiple sacs and polyembryony, features common in apomictic biotypes of *P. pratensis*, suggest that she was dealing with an apomictic form.

MÜNTZING (26), on the basis of progeny tests and chromosome counts on the parent and its matroclinic offspring, concluded that apomixis occurred in *Poa*

(*P. palustris* L.). He postulated (22) that variant plants arising in a diploid-parthenogenetic *Poa* species would be haploids.

ARMSTRONG (8) concluded that fer-



FIGS. 1-8.—Fig. 1, plant 73-19; reproduces primarily by somatic apospory. Figs. 2-7, plants 71-10, 72-7, 73-14, 72-18, 72-23, and 72-6, respectively; reproduce primarily by sexual means. Fig. 8, plants 73-1, 9 and 10; plant on right is parental type.

species. In 1935, KIELLANDER (21) reported the direct development of the E.M.C. into the embryo sac (diploid-parthenogenesis) in *Poa serotina* Ehr.

tilization takes place in *P. pratensis* because he observed paired chromosomes at the heterotypic metaphase of reduction division in the E.M.C., and because

of good pollen germination. He did not find "any regularity as to the position of the functional megaspore in the row, although it was more frequently the inner one farthest from the micropyle" that developed into the embryo sac. He advanced the hypothesis of random assortment of univalents in the homoeotypic division in *P. pratensis* and also suggested that "only pollen grains of the normal chromosome complement function." In this way an aneuploid number might be maintained in a sexually reproducing strain. The "variation in the position of the functioning megaspore in the row of four . . . provided a mechanism for the elimination of megaspores with an abnormal chromosome complement and for the choice of a megaspore containing the normal chromosome complement."

ÅKERBERG (1) figured an aposporous embryo sac located laterally in the nucellus to the collapsed macrospores of the ovule primordium of *P. pratensis*. During the later stages of development only remnants of the E.M.C. or its meiotic products were found.

BROWN (11) suggested that "the extent of occurrence of aneuploid forms in different parts of the world may in some degree account for the diversity of opinion concerning the extent of apomixis within the species" (*P. pratensis*). In 1941, he concluded that "the varying units always tend to group themselves into one of two (morphological) complexes," and that "all its peculiarities point to a probable hybrid origin." Two progenies ($2n = 42$) had 18.2 and 16.7% aberrants. Two other progenies ($2n = 56$) produced 1.5 and 2.97% off-type plants. From these observations he concluded that "although the evidence is not conclusive it does indicate that apomixis

tends to increase as the chromosome number increases."

MÜNTZING (27) observed that the embryo sac of *P. alpina* develops directly from the macrospore mother cell.

ENGELBERT (14) concluded from a study of the development of twin-embryoed seeds in *P. arctica* that the matroclinic appearance of most plants of a given progeny was due to apospory, whereas the aberrants were the result of "sexual (n) embryo" development and occasional cases of fertilization.

TINNEY (40) followed the developmental embryology of apomictic biotypes of Kentucky bluegrass from the macrospore mother cell through to the young ovule. He reported the frequent completion of reduction division, after which the haploid macrospores collapsed. However, collapse sometimes occurred before the division was completed. Simultaneously, a somatic cell (s) assumed the function of the embryo sac and developed a functional embryo(s). KIELLANDER (23) and ÅKERBERG (5) reported similar observations from Swedish biotypes of *P. pratensis*.

KIELLANDER (24) reported that a family ($2n = \pm 72$) of *P. pratensis* was morphologically constant throughout three generations. Differences between members of sets of twins were observed. "Two aberrant forms, a twin with $2n = 40$, and a triplet with $2n = 18$ are described." The eighteen-chromosome plant, after open pollination, produced plants with sixteen to nineteen chromosomes. This plant and its progeny resembled *P. trivialis* L.

ÅKERBERG (6) studied the development of the embryo in emasculated florets of apomictic biotypes of *P. pratensis*. Those florets subsequently pollinated developed endosperm, whereas only proembryos and polar bodies occurred in the

unpollinated. He concluded that pollination is necessary for endosperm and functional seed development.

HÅKANSSON (18) discussed the embryology and fertilization of sexual forms of *P. alpina* from Switzerland and apomictic types from Scandinavia. Any of the haploid macrospores of the sexual plants ($2n = 22-25, 31$) was capable of developing into an embryo sac, with some plants producing more than one. The asexual forms showed no evidence of meiosis, in contrast to the sexual forms. The diploid egg cell produced formed the embryo. One embryo was apparently triploid, indicating fertilization—at least in some apomicts. Endosperm formation occurred “only after fertilization of the polar nuclei had been effected (pseudogamy.” The embryo, in relation to endosperm development, was observed to start development earlier in apomictic than in sexual forms.

EXPERIMENTAL RESULTS.—

EMBRYO-SAC DEVELOPMENT.—The observations reported here were made primarily on plants that showed marked morphological variations among the members of individual progenies. The conclusions are based on an examination of an average of more than fifty fertile florets of each plant.

Although the presence of pollen tubes entering the embryo sac has not been observed, the nature of the development of the embryo sac in florets of plants yielding segregating populations makes it probable that fertilization does take place. A few hours after pollination, pollen tubes were frequently observed in the stylar tissue and in the ovarian cavity adjacent to the micropyle.

The macrospore mother cell, located medially under one hypodermal layer of cells of the ovarian primordium, is de-

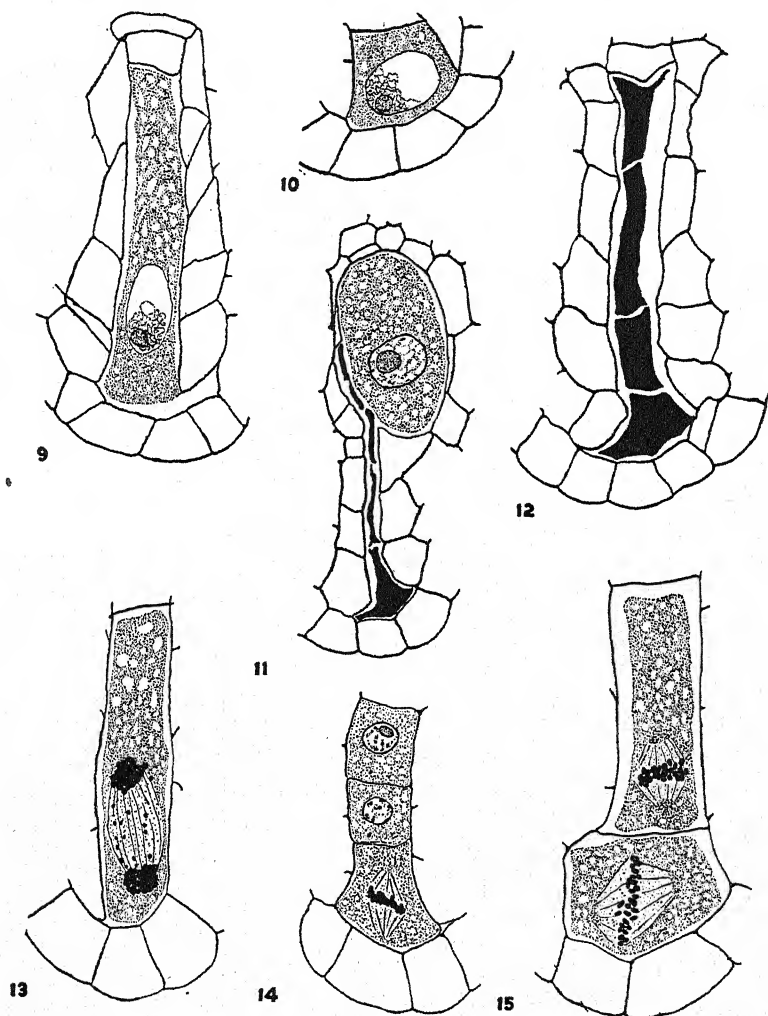
scribed by TINNEY as invariably single and elongated (fig. 9). Although this is usual, it is not uncommon to find macrospore mother cells that are only slightly elongated (fig. 10). In a few cases two sporogenous cells have been found lying adjacent to each other. The development of polyembryonic seeds will not be discussed further, other than to indicate the frequency of multiple embryo sacs in the material studied.

The stages leading up to and through the meiotic divisions are usually somewhat irregular. In some instances dicentric bridges and fragments or chromosomes not included in the figure were observed. At the second division it is not uncommon to find the outer of the two daughter cells with an irregular mass of chromatic material irregularly placed in the cell. The innermost daughter cell usually completes its division, forming haploid macrospores. Another alternative is for both nuclei to divide, with their accompanying cell divisions, and to form a lineal tetrad of haploid macrospores. It is during the foregoing stages that collapse occurs, and a uninucleate nucellar cell may take over the functions of the innermost macrospore (figs. 11, 12). Under these conditions development is from a somatic or diploid cell (somatic apospory). When the macrospores collapse and a somatic cell assumes the function of the embryo sac, development proceeds in the manner described by other workers.

The macrospores of sexual plants sometimes develop according to the “normal type” of embryology. In a fair proportion of cases, the reduction divisions appear quite regular and four haploid macrospores are formed. Figure 13 is illustrative of many of the irregularities observed at anaphase I, and figure 14 shows an excluded body in each of the

two innermost macrospores, whereas in the outermost or micropylar cell most chromosomes are at or near the plate. Although few figures were found wherein the chromosomes in the macrospores could be readily determined, a number of early anaphase figures possessed ap-

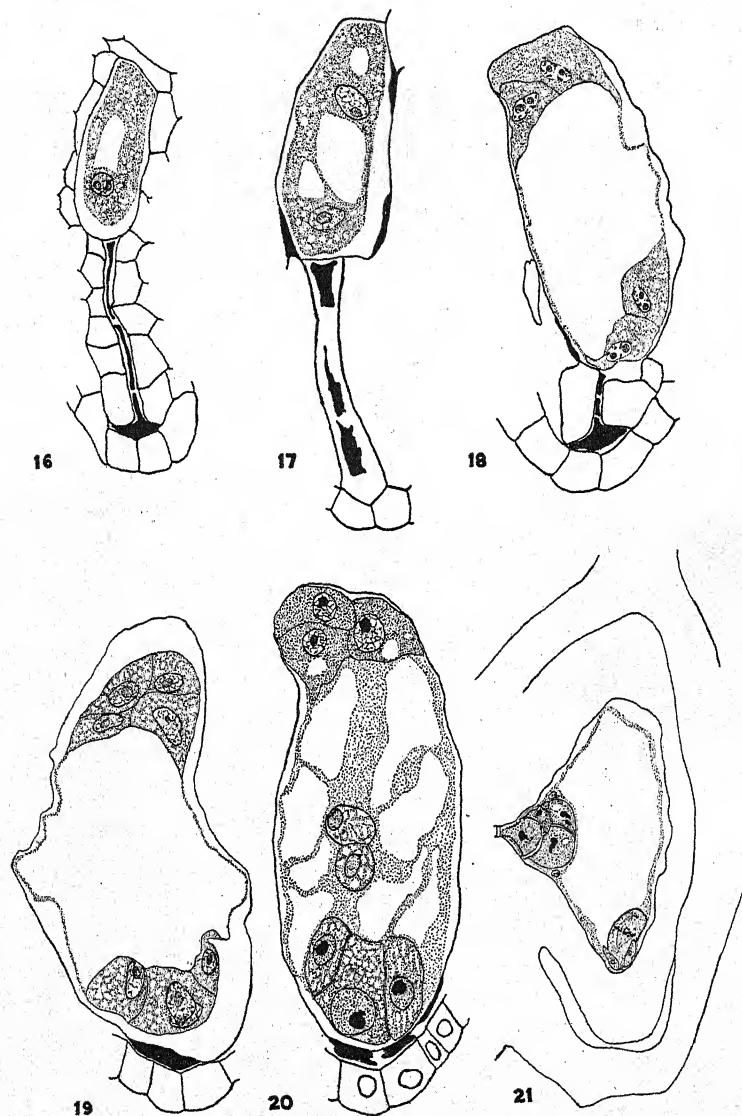
proximately twenty-eight chromosomes (fig. 15). Generally, however, the tetrad is lineal, the figure shown being an exception. Following the formation of the four haploid macrospores, the three outermost ones collapse and eventually disappear, and the innermost one enlarges



FIGS. 9-15.—Camera-lucida drawings of developmental embryology of *Poa pratensis*: Fig. 9, typical elongate macrospore mother cell; plant 70-11. Fig. 10, typical short macrospore mother cell; plant 72-11. Fig. 11, four collapsed macrospores and enlarged somatic cell of somatic aposporous plant that assumes function of innermost macrospore of sexual plants; plant 71-12. Fig. 12, four collapsed macrospores of sterile floret; plant 71-12. Fig. 13, anaphase I of macrospore mother cell; plant 71-12. Fig. 14, triad with delayed division in the outermost cell of dyad; excluded chromosome present in each innermost cell; plant 72-18. Fig. 15, metaphase II; plant 72-16.

and soon becomes univacuolate (fig. 16). The first gametophytic division occurs when this macrospore is about twice its original diameter (fig. 17). This is followed shortly by two divisions, thus

forming a typical eight-nucleate sac (figs. 18, 19). At the division forming the four-nucleate sac, its diameters are about twice those at the preceding division. There is some increase in size by the time



FIGS. 16-21.—Fig. 16, three outermost macrospores collapsed, innermost is univacuolate and uninucleate; plant 72-16. Fig. 17, innermost and functional macrospore is binucleate; plant 72-8. Fig. 18, same, but at four-nucleate stage; plant 72-8. Fig. 19, same, at eight-nucleate stage; plant 70-6. Fig. 20, seven-celled, eight-nucleate stage; plant 70-6. Fig. 21, embryo sac at first zygotic division and after first division of fusion nucleus; plant 72-19.

of the third division, but it is relatively somewhat less than that at the earlier mitoses.

The sac, at the eight-cell stage, has a large central vacuole with four nuclei located in each end of the cell. A protoplasmic belt then forms medially. As the polar nuclei converge toward the center of the sac, bounded on either side by several vacuoles, the synergids become somewhat elongate or pear-shaped, and eventually their cytoplasm may become somewhat fibrillar in appearance (fig. 20). Concurrently the antipodals enlarge and assume a densely staining property and the egg cell becomes somewhat rounded. These three units of the eight-nucleate, seven-celled sac will be discussed separately, although their developmental stages are simultaneous.

The cytoplasmic belt extending from the chalazal to the micropylar end of the sac forms as the antipodals become somewhat rounded in appearance. The cell nearest the funiculus may enlarge to form a haustorium-like structure that perhaps acts as a conductor of food from the provascular strand (fig. 21). The antipodals develop a dense, darkly staining cytoplasmic condition. Scattered throughout these cells are several dark-staining "chromatic" bodies. An occasional division figure was observed. The onset of collapse of the antipodals becomes apparent shortly after the first division of the polar-fusion nucleus. Disintegration and digestion are frequently—but not always—complete by the time the endosperm starts to become cellular.

The polar bodies begin to migrate rather slowly toward each other. Just before the first zygotic division they usually lie free toward the micropylar end of the sac. They then move rapidly toward the egg (or zygote) and fuse when that structure has undergone division. In

some instances the polar-fusion nucleus has completed its first division at the time of the first zygotic division. Fusion appears to occur very near or adjacent to the dividing zygote, or the proembryo, after which there is apparently a rapid migration toward the antipodals. The first division of the polar-fusion nucleus usually occurs just above the antipodals. One of the daughter nuclei passes to the distal side of the antipodals (fig. 21), where it divides again at about the same time as its proximal mate. This is followed by a series of rapid divisions, resulting in a free-nucleate endosperm. At this stage the embryo has increased to about sixty cells and the antipodals have usually almost disappeared.

The need of fertilization for endosperm development in somatic aposporous reproducing biotypes has been referred to by several workers (3, 17, 41, and others). Plant 71-12, which reproduces largely by apomixis (see Progeny tests), is approximately triploid, with ± 40 "bivalents" (see Microsporocytes). At least 378 (*ca.* 375-400) chromosomes were counted in an early anaphase figure of the polar-fusion nucleus.⁵ Since polar bodies in somatic aposporous embryo sacs form from diploid nuclei, they would contribute at least 160 chromosomes to the fusion nucleus. Hence they contributed about 320 members to the anaphase complement, which would leave about 58 (or on basis of *ca.* ± 80) chromosomes unaccounted for except by fertilization of the polar body by an approximately reduced male nucleus. Thirty-seven dividing pairs were counted in about one-fifth of the figure of another meta-anaphase of the fusion nucleus in an embryo

⁵ The polar-fusion nucleus at anaphase (71-12) was loosened from surrounding ovarian tissue on a newly prepared slide. This was drawn off and smeared in thin balsam on another slide, making an approximate count possible.

sac of 71-3. The calculated complement of this figure essentially equaled that of 71-12. These observations are in good agreement with those of ÅKERBERG (6), who concluded that endosperm of a Swedish apomictic biotype of *P. pratensis* contained ± 195 chromosomes.

No dividing endosperm nuclei of sexual plants were found wherein the number of chromosomes could be determined. It appears, however, that they contain about one-third as many chromosomes as do the nuclei of apomictic plants. This assumption is based on the comparative sizes of metaphase and anaphase chromosomes and those of the figures of the two types of plants.

In the micropylar end of the sac the egg enlarges and assumes a loosely granular to alveolar structure. As the egg enlarges, the cytoplasm of the synergids may become somewhat longitudinally fibrillar in appearance. They usually remain distended until the formation of the proembryo and then disappear.

In several plants an enlarged somatic cell was observed in the nucellus adjacent to the embryo sac and distal to the micropyle. These cells were first noted when the sacs were mature. They appeared as though stimulated and might have assumed the function of the embryo sac had the haploid sac failed. These extraneous cells were always uninucleate, and their protoplasts were partially plasmolyzed. They looked already in an early stage of disintegration.

EMBRYO SAC FAILURE.—The divergence in number of seedlings developed from seed in single panicles may possibly be explained by the embryology of the developing floret of the mother plant. In some florets the macrospores form and all collapse (fig. 12). Collapse occurs in other florets at the dyad stage. The meiotic divisions of these show con-

siderable irregularity. In most cases the daughter nuclei of the dyads or macrospore tetrad appear partially organized when collapse occurs. Examination of the surrounding nucellar tissue usually exhibited one of two conditions: (a) no secondary embryo sacs being formed from nucellar cells; or (b) a poorly formed uninucleate sac, similar to those found in most apomictic *Poa* plants. Several factors may have led to these uninucleate somatic sacs being nonfunctional. They were initials when the fertile florets of the spikelet that developed caryopses already had formed cellular endosperm. It would appear doubtful whether they would be able to compete successfully for nutrients with an ovule in a state of storing food.

To determine the significance of embryo sac failure, sectioned material of from twelve to forty florets from each plant was examined. The percentage of functional embryo sacs in florets on each slide was determined. Any embryo sac that had formed and showed no evidence of collapse when fixed was considered functional. For purposes other than this phase of the study, more material was sectioned for some plants than for others. The data were analyzed by analysis of variance to determine whether the variation between plants and that between families was significant. In analyzing data for embryo sac failure, progenies were treated as a whole rather than as morphological groups within progenies. This procedure was followed because the variation within groups appeared to be about the same as that within progenies. Bartlett's chi-square test (X^2) for homogeneity was used to determine whether the mean squares within plants of the several families, and that between plants of the several progenies, were homogeneous (36).

The F values given in table 2 are the ratios of the "between-plant" and the weighted average of the "within-plant" mean squares. Significant F values indicate that the values for the several plants vary more than the average variation within plants. In many cases the X^2 values for the test of homogeneity show that variations within plants are not homogeneous. In order to determine whether any two plants differed significantly in their mean values, either the components of error for these two plants or those components which do not differ significantly among themselves would be used to provide an estimate of the within-plant variation.

Significant F values were found for progenies 71, 72, and 73, indicating differences existing among plants of a family. Likewise, a significant difference was found between families in the amount of sterility. Thus, there is usually a difference in the amount of sterility among plants within families and among families.

The variabilities of sterility among plants of family 72 showed highly significant differences. No differences were found among plants of other families. The P value for comparisons between families exceeded the 5% point.

The percentage of functional embryo sacs was compared also to the frequency of aberrants in progenies developed from seed of these same plants. In florets of families 71-73, with 50% or more functional embryo sacs, the percentage of aberrants decreased as the percentage of embryo sacs increased. This is not true for family 70, where no apparent relationship occurred. Conversely, in those florets having 50% or less functional sacs, the percentage of aberrants was erratic. It appears that as the percentage of functional sacs declines there may be

a tendency toward sexuality. Although this seems to be true for the progenies developed from the original selections, it is not necessarily the case for all plants. For example, plant 71-10 is a marked exception, wherein 84% of the embryo sacs are functional but an entirely diverse progeny was developed from its seed.

Likewise, the uniformity of progenies from plants with low percentages of functional embryo sacs is probably due to seeds formed by the incidence of somatic apospory. There appears to be a fair but not general relation between the percentages of functional sacs and the number of seeds produced.

POLYEMBRYONY.—The percentages of florets of the several plants, and of the plants of families, having multiple embryo sacs are given in table 2. These data are based upon the number of florets possessing functional embryo sacs and not upon the total florets examined. The data are, generally, what might be expected from asexual and sexual plants of this species; that is, plants reproducing asexually tend to produce polyembryonic florets more frequently than do sexually reproducing plants. Plant 71-10 is a marked exception. In the thirty-one florets of this plant having functional embryo sacs, thirteen florets possessed two, and six florets three or more embryo sacs. In most florets, but not all, one embryo sac was well along toward maturity, whereas the other or others were represented by densely cytoplasmic cells in either a uninucleate or a binucleate condition. The supernumerary embryo sacs were therefore primarily somatic aposporous. Sometimes, however, both embryo sacs representing twins were sufficiently well advanced so that one would expect the embryo of the asexual embryo sac to develop as rapidly as that result-

TABLE 2

ANALYSIS OF VARIANCE, CHI-SQUARE TEST FOR HOMOGENEITY, PERCENTAGES
OF FUNCTIONAL AND MULTIPLE EMBRYO SACS,
OF EMBRYOLOGY OF *POA PRATENSIS*

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE	SACS (%)	
			Functional	Multiple
Between plants.....	7	374.0
Within plants 70- 2.....	8	321.7	54.9	25.0
6.....	3	988.2	73.8	15.4
8.....	9	495.7	73.0	16.3
17.....	4	90.2	66.6	27.3
18.....	4	201.5	82.6	31.6
19.....	2	419.4	54.1	23.1
20.....	2	1408.3	77.8	0.0
22.....	3	1031.6	63.0	35.7
Weighted average within plants.....		454.2	69.9¶	6.4
F† =		0.82		
X²‡ =		8.10		
Between plants.....	4	1073.8
Within plants 71- 3.....	4	82.2	93.3	3.6
6.....	4	80.0	96.1	20.0
8.....	1	288.0	46.2	0.0
10.....	4	266.3	83.8	63.3
12.....	5	143.1	88.6	15.4
Weighted average within plants.....		151.0	86.0	20.7
F† =		6.65*§		
X²‡ =		2.7		
Between plants.....	16	3656.0
Within plants 72- 1.....	9	25.5	14.9	0.0
5.....	5	102.6	68.7	18.8
6.....	3	438.9	24.3	0.0
7.....	8	118.3	66.1	0.0
8.....	16	378.4	66.6	0.0
9.....	5	125.1	10.0	0.0
11.....	11	399.1	51.2	0.0
12.....	4	33.8	12.5	0.0
13.....	7	689.4	47.5	0.0
14.....	4	897.8	88.8	9.4
16.....	8	892.3	50.0	0.0
17.....	5	401.9	75.6	3.9
18.....	5	425.1	64.6	0.0
19.....	8	624.6	46.7	0.0
22.....	5	224.2	75.0	0.0
23.....	15	804.2	34.9	0.0
24.....	13	205.0	67.3	0.0
Weighted average within plants.....		417.9	49.7	0.1
F† =		8.75**§		
X²‡ =		44.6**§		

¶ Plants 11 and 22 with 88.9 and 61.2% functional embryo sacs included in weighted average.

† F = ratio of between- to within-mean squares.

‡ X² = test of homogeneity of within-plant and within-family mean squares.

§ * = significant 5% level; ** significant 1% level.

TABLE 2—Continued

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE	SACS (%)	
			Functional	Multiple
Between plants.....	10	2497.6		
Within plants 73-	9	499.4	83.4	12.9
1.....	14	451.6	80.3	2.7
2.....	3	50.7	93.9	9.7
3.....	17	473.2	56.6	1.6
4.....	10	649.0	54.3	7.3
5.....	8	106.4	84.8	13.2
6.....	8	886.2	53.2	4.3
7.....	10	531.4	52.0	16.1
8.....	13	248.5	72.1	0.0
9.....	9	570.4	80.9	10.9
10.....	9	263.4	80.0	31.8
Weighted average within plants.....		449.9	71.3	6.9
$F\ddagger =$		5.55*§		
$X^2\ddagger =$		15.2		
Between families.....	3	11872.7		
Between plants within families.....	37	2442.8		
$F\ddagger =$		4.86**§		
$X^2\ddagger =$		9.21*§		

ing from gametic union. It is readily possible that at least some of the aberrants in the diverse progeny produced by this plant were the result of a somatic aposporous embryo sac being fertilized and would consequently give rise to a plant differing morphologically from its maternal parent.

MICROSPOROCTES

LITERATURE REVIEW.—

Reference is made here only to papers wherein the meiotic divisions in *Poa* species are discussed. KATTERMANN (20) reported that *Poa caesia* J. E. Smith (*P. glauca* Vahl.) was meiotically irregular and that univalents sometimes divided during the first division and occasionally failed to divide.

MÜNTZING (26) observed irregularities during meiosis in *P. alpina*. He noted univalents at heterotypic metaphase, as well as multivalent groups. At anaphase

I, the univalents split and at anaphase II lagging occurred.

RANCKEN (35) found at least two tetravalent groups in pre-metaphase figures of *P. pratensis*. Pairing was in the form of rings, crosses, and rods, indicating terminal as well as interstitial chiasmata. He did not observe the laggards being reorganized into micronuclei and thus concluded that the excluded chromatic material was reabsorbed into the cytoplasm. Neither did he observe hexads or octets, or other sporocyte groups of more than four members. In addition to the lagging univalents and fragments, he recognized several inconspicuous extraneous bodies.

FLOVIK (16) reported the meiotic divisions of *P. alpigena* type *iantha* ($2n = \pm 77$) and *P. alpigena* type *domestica* ($2n = 83$) to be "generally extremely irregular." Well-organized metaphase plates were observed only occasionally, and laggards were numerous. Micronu-

clei were not common at interphase, indicating that most laggards reached the poles before formation of the daughter nuclei. Laggards of the second division did not reach the poles in time to be included in the quartet nuclei. This often resulted in pentads, hexads, etc. Although individual species differ somewhat in detail from the preceding, FLOVİK observed generally irregular meiosis in *P. alpigena* var. *vivipara* (Malmgr.) Scholander ($2n = 42 + 4ff$); *P. glauca* ($2n = 72$); and *P. arctica*, and *P. arctica* var. *vivipara* (Malmgr.) Scholander ($2n = 56$).

MÜNTZING (27) found that in meiotic divisions in a haploid ($n = 18$) plant of *P. pratensis*, 76% of metaphase I figures were "perfectly regular, 1 to 3 univalents being present in the other cells." In 100 A_1 cells, where the division was 18-18, there were no univalents in ninety-five of the cells. At interphase only three cells had laggards. He also observed a chromatin bridge and a fragment.

BROWN (12) concluded from his study of the same species that "pairing is for the most part, by bivalents, although univalents, trivalents, and quadrivalents have been observed in all cells examined." The univalents, although lagging at anaphase I, "always reach the poles in time to be included in one or the other of the polar groups." He also observed that some plants were "heterozygous for an inversion or duplication as indicated by the occasional occurrence of dicentric chromatids. . . ."

EXPERIMENTAL RESULTS.—

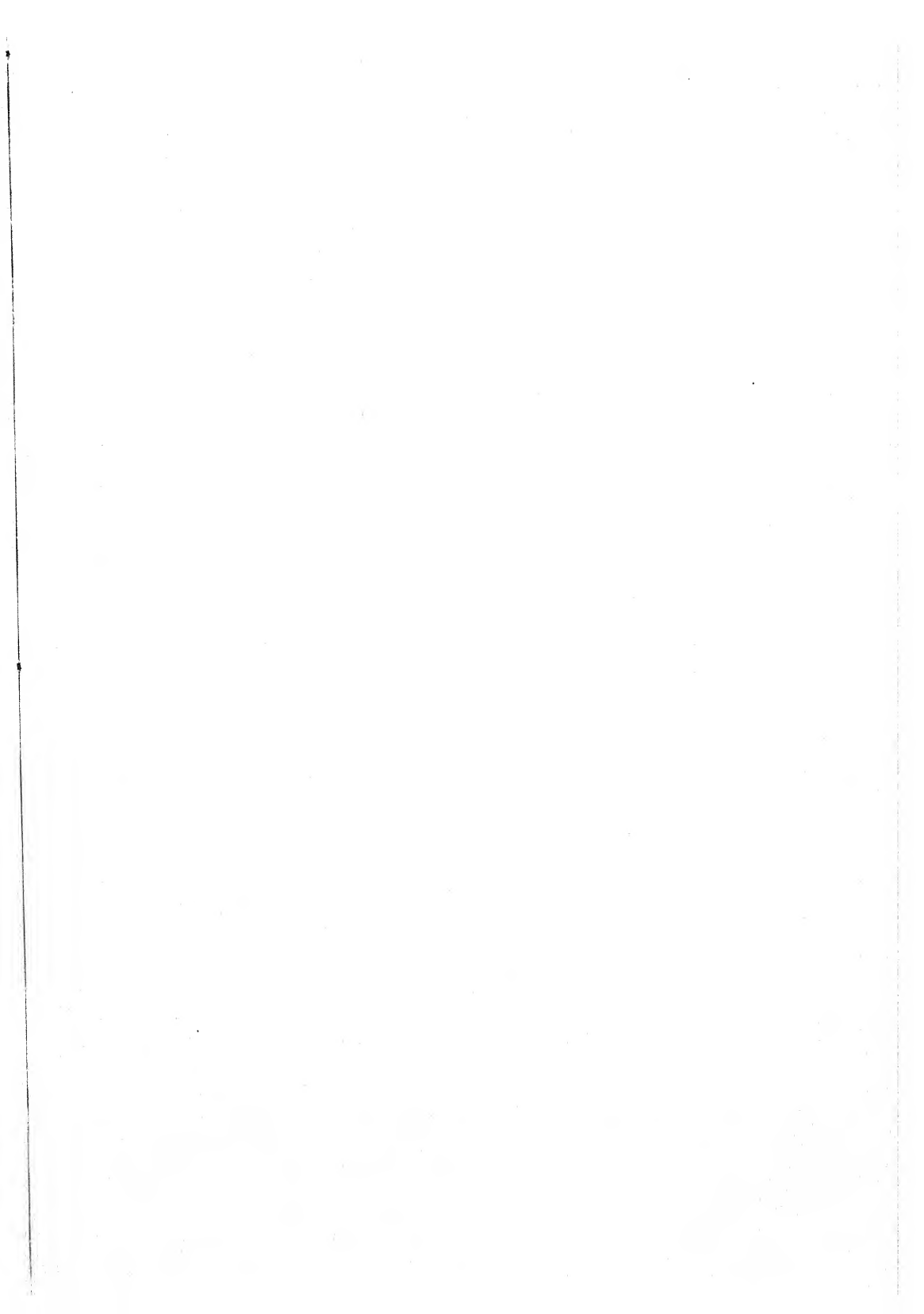
The microsporocytes of twenty-seven individuals were examined. Three plants were first taken at random. One hundred observations were made on these plants at each of the M_I , A_I , T_I ,⁶ dyad, and quartet stages of development. Twenty-

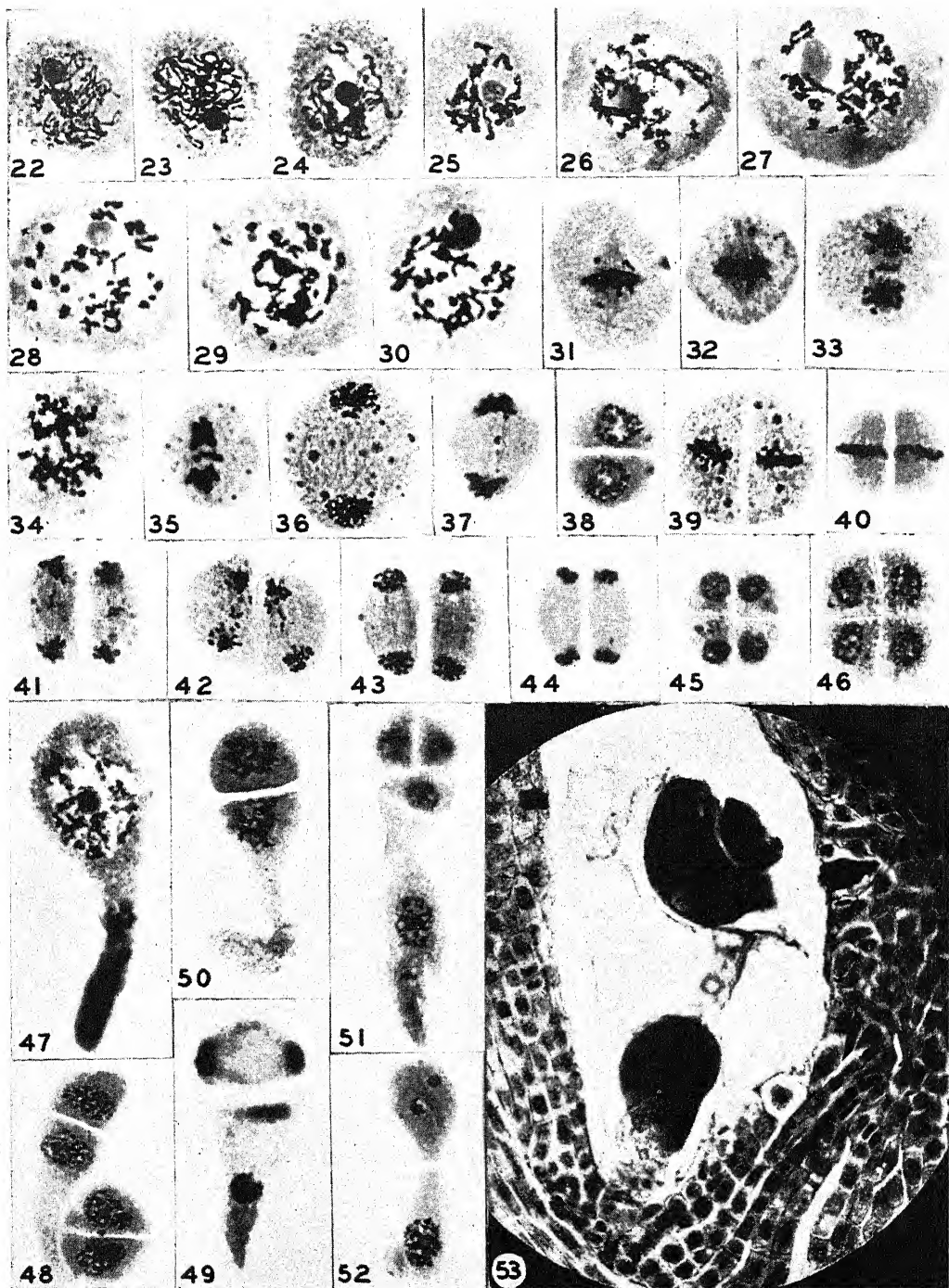
five observations gave sufficient accuracy for comparative purposes. In the section dealing with embryology it was indicated that the plants of segregating populations examined had about twenty-eight bivalents. Microsporocytes of two plants reproducing apomictically had approximately forty bivalents. Since their irregularities are of about the same frequency as the $2n = \pm 56$ chromosome plants, they are not considered separately.

The sporocytes usually contained, in addition to frequent laggards, globular inclusions that took varying amounts of aceto-carmin stain. Representative illustrations are given in figures 22-52. Generally, there was sufficient difference between the staining properties of chromatin and that of the globules to make separation readily possible; other microsporocytes could be studied only with difficulty. The globules may be evident at any stage from prophase through the quartets. They are, however, most conspicuous in anaphase and telophase figures. No plant was entirely free from globules at all stages. The ranges in percentage of cells with globules were at M_I 4-80%, at A_I 4-88%, at T_I 8-80%, at dyad 0-64%, and at quartet 0-44%. Although RANCKEN's (35) reference to foreign bodies in *P. pratensis* microsporocytes is indefinite, it is readily possible that the structures he referred to were similar to those figured here.

There were approximately twenty-eight bivalents in most of the material studied. Incident to this number there is also a marked tendency for individual members of the complement to bunch together, making analysis difficult in most figures. It was concluded, therefore, that a determination of the frequency of lag-

⁶ The symbols M_I , A_I , and T_I are used for metaphase I, anaphase I, and telophase I, respectively.





[Legends for figures 22-53 on facing page]

gards was sufficient for this study. The data from such an examination are given in table 3. These, together with representative forms shown in figures 22-52, are illustrative of meiosis in the material examined.

During the prophase stages the chromatids appear to be normally paired (figs. 22-24). At diakinesis many of the figures exhibited disturbances. Some of the chromosomes shown at 5:00-6:00 o'clock in figure 28 are unpaired. Chromatin strands were evident in figures 27-29. Whether such figures are characteristic of the plants or due to an early stage of necrosis of the sporocytes was not determined. Figure 27 contains also an association of a ring of four chromosomes. Other multivalent groups of three and four chromosomes may be seen in several of the figures.

The data for lagging univalents at M_I , A_I , T_I , dyad, and quartet stages were analyzed similarly to those for embryo sac failure. The results are summarized in table 3. The mean number of laggards at each of the above-mentioned stages of development are also included. In some cases the number of laggards at M_I is somewhat lower than that at A_I . It is likely that a part of this difference may be due to some chromosomes being considered on the plate when actually they were not. There is also the possibility that all chromosomes were not included in the spindle, or that chiasmata failed

to terminalize, thus leaving non-congressing members in the plate region. The ranges of microsporocytes without laggards were at M_I 0-32%, at A_I 0-20%, at T_I 0-56%, at dyad 12-100%, and at quartet 16-100%. The data show that differences are not entirely consistent for all stages. Significant differences were found in the meiotic behavior between plants within several families for all stages except A_I of families 70 and 71, and the dyad and quartet stages of the latter progeny. No differences were found between the mean values of the several families for any of the characters.

Significant differences among plants for the within-plant mean squares were determined for all stages of development (excepting M_I) of progeny 70, M_I , T_I , and dyad of progeny 71. With certain exceptions, values are usually highly significant, indicating heterogeneity; thus certain plants are more variable than others. Highly significant X^2 values for the average mean squares within plants between progenies were found, whereas the X^2 values between plants within families were not significant.

In addition to these meiotic irregularities, bridges frequently—but not always—accompanied by an acentric fragment were observed. Eighteen such bridges were seen. Three occurred in microsporocytes of plants 70-8, 71-3, and 73-2. Two occurred in sporocytes of 71-8, 72-9, and 72-14. There was one each in 70-

FIGS. 22-53.—Sporocytes of *Poa pratensis*: Fig. 22, prophase; plant 70-22. Fig. 23, prophase; plant 70-22. Fig. 24, prophase; 73-11. Fig. 25, diakinesis; 70-22. Fig. 26, diakinesis; 73-14. Fig. 27, diakinesis; 73-14. Fig. 28, diakinesis; 73-14. Fig. 29, diakinesis; 73-14. Fig. 30, diakinesis; 73-14. Fig. 31, metaphase I; 73-21. Fig. 32, metaphase I; 72-6. Fig. 33, anaphase I; 70-22. Fig. 34, anaphase I; 72-16. Fig. 35, anaphase I; 72-6. Fig. 36, telophase I showing globules; 70-22. Fig. 37, telophase I; 73-21. Fig. 38, dyad; 73-21. Fig. 39, metaphase II; 70-22. Fig. 40, metaphase II; 73-21. Fig. 41, late anaphase II; 70-22. Fig. 42, late anaphase II; 70-22. Fig. 43, telophase II; 70-22. Fig. 44, telophase II; 72-6. Fig. 45, quartet; 70-22. Fig. 46, quartet; 73-11. Fig. 47, anomalous sporocyte at diakinesis; 72-16. Fig. 48, anomalous and normal dyads; 73-21. Fig. 49, anomalous sporocyte at telophase II; 73-21. Fig. 50, anomalous sporocyte at early anaphase II; 70-22. Fig. 51, anomalous quartet with secondary division figure in lower projection; 70-22. Fig. 52, anomalous sporocyte with bivalent, univalent, and nucleolus in upper cell; 73-1. Fig. 53, anomalous embryo sac with misplaced antipodal; 70-22.

TABLE 3
AVERAGE LAGGARDS PER SPOROCYTE, ANALYSIS OF VARIANCE, AND
CHI-SQUARE TEST FOR HOMOGENEITY IN *POA PRATENSIS*

SOURCE OF VARIATION	METAPHASE I		ANAPHASE I		TELOPHASE I		DYAD		QUARTET	
	I	Ms	I	Ms	I	Ms	I	Ms	I	Ms
Between and within plants, for the several families										
Between plants.....	31.53	19.62	50.8	14.3	5.3
Within plants:										
70- 8.....	4.7	9.04	3.9	13.10	2.5	3.18	2.4	4.69	1.2	2.33
11.....	2.6	11.75	6.4	8.08	5.1	14.49	1.6	3.25	1.6	4.75
13.....	5.5	6.25	4.2	3.04	2.8	4.48	1.1	1.69	0.9	1.33
17.....	6.2	6.63	4.1	3.17	2.8	2.90	1.2	1.39	1.3	2.46
18.....	4.5	5.92	4.8	7.75	5.3	10.63	3.3	5.14	1.3	2.63
20.....	4.8	7.04	5.2	6.87	3.0	4.63	2.1	4.61	2.2	4.47
22.....	4.1	6.71	4.2	4.33	1.3	2.64	2.0	3.08	0.8	1.61
Weighted averages within plants.....	4.6	7.62	4.7	6.63	3.3	6.06	1.9	3.41	1.3	2.80
F \dagger =		4.14**†		2.96		3.38**		4.20**		1.90
X ² †(6 df) =		4.5		20.6**		31.9**		17.0**		16.0*
Between plants.....	22.41	7.80	18.17	3.70	2.73
Within plants:										
71- 3.....	4.6	3.08	3.0	3.25	1.8	2.73	1.5	2.18	1.0	1.08
8.....	2.8	3.12	2.4	1.74	0.8	0.94	0.6	0.92	0.2	0.27
10.....	2.4	3.54	2.7	6.29	2.0	2.46	0.9	1.58	0.8	2.14
12.....	3.2	3.48	3.7	5.31	2.8	2.14	0.9	1.16	0.6	0.75
Weighted averages within plants.....	3.3	3.31	2.9	4.15	1.9	2.06	1.0	1.46	0.7	1.06
F \dagger =		6.77**		1.88		8.80**		2.54		2.57
X ² †(3 df) =		0.60		10.6*		7.2		5.0		12.5**
Between plants.....	27.31	43.81	22.59	18.76	5.1
Within plants:										
72- 1.....	4.2	2.25	3.1	4.44	2.0	5.96	1.4	2.25	1.3	2.06
5.....	5.3	6.40	6.2	7.75	4.1	4.26	2.6	3.50	1.8	1.08
7.....	2.0	2.83	2.4	3.50	2.1	5.07	1.6	4.92	1.0	2.71
9.....	5.2	20.58§	4.2	4.31	2.4	3.75	1.2	1.39	1.2	2.39
13.....	3.0	2.29	3.4	2.76	3.0	0.83	0.0	0.04
14.....	3.9	3.11	4.1	4.74	1.4	1.99	1.5	1.76	0.4	0.42
16.....	3.0	6.63	6.3	19.96§	4.1	20.56§	3.8	8.23§	1.2	1.17
17.....	3.9	4.49	3.8	6.67	2.3	3.54	2.2	2.81	1.2	0.88
23.....	4.0	3.63	5.0	4.88	2.7	2.38	1.8	1.81	0.2	0.36
Weighted averages within plants.....	3.8	5.81	4.3	6.56	2.7	5.37	1.8	3.33	0.8	1.23
F \dagger =		4.70**		6.68**		4.20**		5.63**		4.15**
X ² †(8 df) =		57.9** (15.3)		38.5** (8.8)		77.8** (27.5)		31.6** (14.0**)		41.8** (41.4**)

† F = ratio of the between- to the within-mean squares.

† X² = test of homogeneity of the within-plant and within-family mean squares.

* = significant at 5% level; ** significant at 1% level. § Removed from calculation for X² value given in parentheses.

TABLE 3—*Continued*

SOURCE OF VARIATION	METAPHASE I		ANAPHASE I		TELOPHASE I		DYAD		QUARTET	
	I	Ms	I	Ms	I	Ms	I	Ms	I	Ms
	Between and within plants, for the several families									
Between plants.....	98.53	31.33	32.58	6.50	16.50
Within plants:										
73- 1.....	6.7	10.04	4.0	1.33§	2.2	2.97	1.4	1.83	2.4	2.91
2.....	7.0	5.04	5.2	5.23	3.1	5.03	1.2	1.69	0.12	1.61
5.....	3.8	2.69	6.8	6.69	4.8	9.03§	2.4	4.83§	2.6	4.24§
11.....	3.2	3.48	3.2	3.17	2.2	1.73	0.9	1.28	1.0	1.54
14.....	2.0	4.13	4.9	8.78§	4.0	5.21	0.8	0.83	1.0	1.46
15.....	6.4	11.42§	4.2	3.98	1.6	2.42	1.3	1.81	1.0	2.00
21.....	3.8	3.17	4.7	3.46	2.4	1.68	1.3	2.46	0.4	0.67
Weighted averages within plants.....	4.7	5.71	4.7	4.66	2.9	4.01	1.3	2.11	1.4	2.06
F η =	17.26**		6.72**		8.13**		3.08**		8.01**	
X 2 †(6 df) =	24.3**		23.3**		28.7**		51.8**		22.9**	
	(14.8*)		(4.4)		(14.6*)		(7.6)		(12.4)	
	Average within plants for several families									
		Ms		Ms		Ms		Ms		Ms
X 2 †(3 df) =		18.8**		12.0**		34.9**		28.6**		38.6**
	Between families and between plants within families									
	df	Ms	df	Ms	df	Ms	df	Ms	df	Ms
Between families...	3	69.26	3	84.5	3	43.90	3	34.6	3	20.10
Between plants within families:										
70.....	6	31.53	6	19.50	6	50.83	6	14.30	6	5.32
71.....	3	22.40	3	7.80	3	18.17	3	3.70	3	2.73
72.....	8	27.31	8	43.81	8	22.59	7	18.76	8	5.10
73.....	6	98.53	6	31.33	6	32.58	6	6.50	6	16.52
Weighted averages within plants.....		46.35		29.52		31.99		12.14		7.83
F η =	1.50		2.86		1.37		2.85		2.57	
X 2 † =	3.9		2.69		1.46		3.05		4.13	

17, 71-10, and 73-21. Thus at least nine of the twenty-seven plants studied may have been heterozygous for an inversion or duplication.

These observations upon quartets differ from those of BROWN (12). He reported seeing no micronuclei in quartets, whereas in the data reported here only one plant was entirely free from this irregularity. In only three of the plants examined were 75% or more of the sporocytes without micronuclei.

Multiple sporocytes were observed in plants 72-5, 72-9, and 73-11. These included two triads, one pentad, five hexads, and one heptad. The numerous meiotic irregularities would lead one to assume that multiple sporocytes would be observed more frequently.

There does not appear to be any direct relationship between the frequency of laggards during meiosis in microsporocytes and the breeding behavior. Neither does this frequency of laggards appear to be directly related to embryo sac collapse.

ANOMALOUS STRUCTURES

Certain anomalies have been observed of which only passing mention will be made.

A microsporocyte approximately at diakinesis is shown in figure 47. A cytoplasmic "tube," most of which is out of focus, is also evident. Two sporocytes at M_I united by a cytoplasmic tube were also observed. That some of such anomalous sporocytes complete their division is evidenced by the dyad shown in figure 48, early A_{II} in figure 50, telophase $_{II}$ in figure 49, and the quartet stage in figure 51. The latter photograph shows a secondary division figure involving approximately four chromosomes in the lower left projection. Likewise, the "sporocytes" shown in figure 52 are peculiar,

since the lower cell was at one of the prophase stages. The upper cell shows a nucleolus, a bivalent, and a univalent. Such a configuration may represent a variation of the abnormalities, described earlier in this paragraph. If so, three laggards were apparently left in the protuberance; hence, the figure of a bivalent and univalent, neither of which is in direct association with the nucleolus. The origin of these structures is not clear. There is no evidence that they arise in any particular portions of the thecae. Conversely, in plant 73-21, where such structures were relatively common, they were observed twice in medial portions of unbroken sporogenous tissue but not in terminal or end positions.

Figure 53 shows a longitudinal section of an ovule. One of the antipodals of the embryo sac is located in the micropylar end rather than laterally toward the chalaza of the developing ovule. A normal cytoplasmic membrane was evident surrounding the displaced antipodal. The floret shown was probably apomictic, since it may be observed that a pro-embryo of approximately sixteen to twenty cells had formed prior to the first division of the fusion nucleus—which cannot be seen in the plane photographed. The anomaly figured here is essentially similar to that recently reported by HÅKANSSON (18).

Discussion

The data that have been presented pertain primarily to processes consequential to apomixis in *Poa pratensis*. They do not explain causal phenomena. Throughout the data the cytogenetic instability of closely related plants has been evident. There have been marked and inconsistent variations among the different plants in each of the phases of the life cycle studied.

Classification of plants on a morphological basis was found to be unrelated to the development of seedlings in progeny tests. Although plants were considered to be morphologically similar, differences occurred in both number and vigor of seedlings. The data suggest that these characters are independent and that it does not necessarily follow that vigorous plants will produce numerous or vigorous seedlings.

There was, generally, no direct relationship between plant morphology and breeding behavior. Individual plants, either weak or strong, might produce a morphologically constant or a segregating progeny. Hence, it is not possible to predict, or valid to assume, that morphologically similar plants will behave in a concomitant manner as expressed by breeding behavior. Neither does there appear to be a direct relationship between the number of seedlings produced, or their vigor, and the uniformity of a random population of mature plants grown from those seedlings.

Analysis of data concerning the meiotic divisions in microsporocytes aligns itself with the foregoing statements. For the most part, significant differences in irregularity among plants of the same progeny were determined. Similar differences also were ascertained among related progenies.

The incidence of the collapse of developing macrosporocytes is suggestive of not being related to plant type or other characters. A complicating factor enters, however, with the introduction of an asexual type of reproduction. The limited data available at a high incidence of embryo sac collapse are inconclusive, but they are suggestive, none the less, that no direct relationship exists between this factor and somatic apospory in *Poa*. With the introduction or assumption of

apomixis, the picture is largely hidden. However, as progenies become more uniform there is also an increase in the frequency of functional embryo sacs.

Several workers have suggested that *P. pratensis* is of hybrid origin (12, 23, and others). Present studies lend strong support to the assumption. The meiotic irregularities observed in microsporocytes are essentially similar to those reported for known grass hybrids (33, 34, 29, 19, 25, and others). Concomitantly, the meiotic irregularities in the developing macrospores are sufficiently great to lead to almost complete sterility in some plants. This sterility and the meiotic irregularities in the sporocytes suggest that somewhat remotely related genomes may have been involved in the synthesis of the species. As a result of his study of a haploid twin *P. pratensis* plant, KIELLANDER (24) suggested that *P. trivialis* may have been involved as one of the species in the cross. Among twins isolated by this writer is one $n = 14$ (unpublished data). This plant bore no resemblance to *P. trivialis*.

It has been shown by several workers that progenies developed from field collections are predominantly constant morphologically. Occasionally such a collection produces a heteromorphic progeny when grown as spaced plants. The aberrants occasionally reproduce apomictically, but predominantly they develop heterogeneous progenies, many plants of which are weak and perhaps would soon disappear from a mass-seeded population. Some, however, are sufficiently vigorous to appear able to compete successfully with their usually more vigorous, apomictic types. The possibility of reduced and unreduced female gametes being fertilized also exists (18). These unreduced gametes, which have been developed from a somatic cell, may

be either eu- or aneuploid. When they are fertilized, biotypes of chromosome numbers different from those of the parents arise. Whether or not *Poa* pollen of aneuploid chromosome number is functional is not definitely known. If so, further aneuploidy may be promulgated. It is only when complementary sets of chromosomes are joined that constancy of cytological behavior might be expected. Judging from the data presented, this is only occasional, and hence there occurs in the species a range of chromosome numbers varying from eighteen to well over 100 (9, 38, and others).

The fact that there are frequent meiotic disturbances of *Poa* resulting in reduced fertility in apomictic types is overcome by the asexual form of reproduction assumed by the species. It is only when factors for apomixis (the development of somatic "gametophytes") are absent that a reasonably accurate measure of the genetic make-up of *P. pratensis* as a probable allopolyploid species is possible.

There is good agreement between the data presented and that of MÜNTZING and MÜNTZING (28). They were able to synthesize a "new constant biotype of the basic chromosome number of the genus" by crossing certain strains of *Potentilla opaca* L. onto segregates of *P. argenticola* L. Principal analogies between plants and progenies from controlled crosses of sexual, partially apomictic and apomictic, biotypes of *Potentilla* and those from open-pollinated *Poa* plants may be summarized as follows: (a) New constant lines are established from aberrants with approximately the diploid and triploid chromosome number; both types are apomictic, one arising by an internal genetic change (28) and the other by fertilization of an aposporous egg cell. (b) The poor seed set in some

Poa plants is essentially similar to that known for known interspecific crosses in *Potentilla*. (c) Irregularity in meiosis is due to hybridity, whereas constancy in morphological type is due to apomixis. (d) The occasional occurrence of sexually reproducing florets in apomictic plants, and its converse, result in the diversity of naturally occurring populations of these species.

Summary

1. Germination studies carried on in soil in pans, and in a seed germinator, indicated wide average differences among plants of *Poa pratensis* in the number of seeds set and also in the viability of such seeds. Although germination percentages of a few plants were less than 10%, most plants produced seed of good viability. The poor seed set of some plants was due to frequent collapse of the developing macrosporocyte during or just after meiosis. Significant differences in the amount of such sterility was determined for between-progeny comparisons. Plants within a given progeny were significantly different among members in one progeny, that is, 72.

2. Forty-four progenies from as many plants of four related parental families were grown. The frequencies of aberrant plants among the second-generation individuals varied from zero to 100%. There did not appear to be any direct relation between the morphological characteristics of parent plants and the breeding behavior of their progenies. This also appears true for other characters, such as seed set, germination, etc.

3. Globular inclusions occurred in the developing microsporocytes of all plants. These were particularly prominent during anaphase and telophase stages, although they were evident in all stages from prophase through the quartets. Highly significant differences generally

were found in microsporocytes in the frequency of laggards among plants, but not always.

4. Certain anomalies among developing ovules and microsporocytes were observed and figured. Their origin was not determined.

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GROWTH AND DEVELOPMENT IN RANGE GRASSES. V. PHOTO- PERIODIC RESPONSES OF CLONAL DIVISIONS OF THREE LATITUDINAL STRAINS OF SIDE-OATS GRAMA¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 565

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Introduction

Earlier work (6) has shown that twelve geographic strains of side-oats grama (*Bouteloua curtipendula* [Michx.] Torr.) are decidedly differentiated in their photoperiodic responses, as judged by their behavior over a 2-year period on Chicago natural daylength and on constant daily photoperiods of 9, 13, 16, and 20 hours. Three strains from southern Texas and Arizona apparently consist largely of intermediate- or short-day plants, while a strain from North Dakota is probably made up chiefly of long-day plants. The other eight strains, from Nebraska, Kansas, Oklahoma, and New Mexico, seemed

to include numerous long-day individuals, although the length of the critical photoperiod for the "late" plants decreased with decrease in latitude of origin. The Oklahoma and New Mexico strains showed considerable diversity in response within each strain on each treatment, and may include both intermediate- and long-day plants. These studies and conclusions were based on populations of twenty to fifty individuals of each strain on each of the five treatments.

It seemed desirable to determine somewhat more precisely the degree of diversity in photoperiodic response within some of these strains, and to narrow the range of treatments so that the results might better indicate the length of criti-

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cal photoperiods and the practical importance of photoperiod in the seasonal and latitudinal adjustment of these strains. Accordingly, clonal divisions of twelve selected individuals from each of three strains were grown during 1944 on Chicago natural daylength and on constant photoperiods of 13, 14, and 15 (or more) hours. Thus, genetically identical populations were grown on each treatment.

The necessary background of related studies by other investigators, the reasons for this general type of investigation and choice of species, and the values which may be derived, have been discussed in previous papers (5, 6) and will not be repeated here.

Material, methods, and environmental conditions

Strains 1, 5, and 12 of the previous report (6) were selected for investigation in 1944, since they represent the extremes and approximate means of latitudinal origin (approximately $29\frac{1}{2}$, $35\frac{1}{2}$, and $46\frac{1}{2}$ degrees N) and range of response of the twelve strains grown in 1942-43. Strain 1 originated at San Antonio, Texas; strain 5 at El Reno, Oklahoma; and strain 12 at Cannonball, North Dakota. On April 16, 1944, twelve individuals from each strain were picked from populations of 2-year-old plants which had been grown chiefly on Chicago natural photoperiod until late December, 1943. These individuals were chosen so as to sample the variation previously shown by these populations in vegetative and reproductive habits, including early and late flowering (or nonflowering), on Chicago natural photoperiod.

In the autumn of 1943, plants of strains 5 and 12 had become dormant in a warm greenhouse, dying back to within 2-3 cm. of the ground, strain 1 to a

lesser degree, apparently in response to decreasing light intensity and photoperiod (6). In late December these plants were given supplementary illumination to provide a 16-hour photoperiod. They grew slowly in January, and by mid-February new foliage averaged 15-20 cm. tall. On March 29 these plants were again placed on natural photoperiod, when they ranged from 30 cm. (strain 1) to 20 cm. (strain 12) in height. No flowering and little culm elongation had occurred by April 16, when the selected plants were clipped back to an average foliage height of 7.5 cm. at the time of transplanting and division.

Each selected individual was divided into four equal pieces, the old roots being pruned to a length of 2.5-5 cm. The divisions were planted singly in 8-inch unglazed clay pots filled with a silt-loam soil. This volume of soil was apparently adequate to allow vigorous development of each division, so that it is unlikely that either the vigor of flowering or the length of the flowering season was limited by this factor. It is probable that the effects of clipping and root pruning may have somewhat delayed subsequent floral initiation, since one of the reserve unclipped individuals of strain 5 on natural photoperiod exerted an inflorescence on June 1. This may be compared with the appearance of the first inflorescence on July 22 in the clipped plants on natural photoperiod in the experiment, while all other clones of this strain and series flowered only after August 2. In the 13-hour series, however, all exerted their first inflorescences between June 26 and August 5. This suggests that possibly some of the experimental plants of strain 5 on natural photoperiod might have flowered much sooner if they had not been relatively defoliated during exposure to effective photoperiods ranging

from 14 to 15 hours in late April and early May—possibly more favorable to them for floral initiation than the longer ones of June. In previous experiments unclipped plants of strain 12 on natural photoperiod exerted inflorescences as early as May 15, in contrast with June 26 for the clipped plants of the experiment. No plants of strain 1 at Chicago, however, have flowered in spring or summer on natural photoperiod.

The meristems of a large number of unclipped plants comparable with those selected for clonal division were examined with a dissecting binocular microscope on April 16. No inflorescence primordia were found in any of the strains. It is thus probable that inflorescences appearing in 1944 in both clonal divisions and reserve plants were initiated after April 16 while on the photoperiods on which they were placed on that date, and not during some preceding period. It had been shown previously (5) for a different strain that clipping apparently removes the possibility of much after-effect of previous photoperiods. This would seem to be even more the case when floral primordia, which might escape clipping, are not already present, since it has been shown repeatedly for certain species (1, 2) that the photoperiodic stimulus to flower is exerted through the leaves and then transmitted to the meristems.

The four divisions of each individual of each strain were distributed to four trucks or benches to receive photoperiods of 13, 14, and 15 hours, and Chicago natural daylength (N series). The 13- and 14-hour series, on adjacent movable trucks, received natural daylight for 9 hours between 8:00 and 5:00 P.M. (CDT) but were rolled into ventilated light-proof sheds for the remainder of the 24 hours. The 13-hour series received 4

hours of artificial light from 5:00 to 9:00 P.M., while the 14-hour series was given 5 hours between 5:00 and 10:00 P.M. These treatments thus differed by only one hour of artificial light. The intensity of supplementary light, from 200-watt Mazda lamps mounted in individual reflectors, varied from 100 to 180 foot-candles at average foliage height. The 15-hour series on a stationary truck in the same room received supplementary light after sunset, when necessary, to provide a photoperiod of 15 hours or more. Between June 2 and July 19 this series was left on natural daylength only. Thus for a time in late spring and early summer it was on photoperiods ranging between 15 and 16 hours, but after August 1 the effective photoperiod was little more than 15 hours. The N series was in a separate greenhouse room similar in temperature and natural light intensity conditions to that containing the other three series.

Responses on Chicago natural daylength may be usefully compared with behavior under field conditions and with the results obtained in similar series in the two preceding years. Thirteen-hour treatments were also used previously. This photoperiod is near or below the lower limit of the photoperiodic range on which these strains flower under natural conditions. The North Dakota strain is subjected to photoperiods of 14–17 hours or more during most of its natural growing season, while effective natural daylength in Texas and Oklahoma ranges between 14 and 15 hours (possibly 15½ in Oklahoma) for a much shorter time in late spring and early summer. Maximum photoperiods (sunrise to sunset at the summer solstice) for the three strains are approximately 14 hours (Texas), 14 hours and 35 minutes (Oklahoma), and 15 hours and 50 minutes (North Dako-

ta). Thus critical photoperiods of significance in the native environments for either short- or long-day plants of these three strains might be expected to fall very near or within the relatively narrow range of treatments of this experiment, although the status of intermediates would not be completely clarified.

Growth was resumed fairly rapidly after transplanting by most of the clonal divisions. Most of them, with the exception of strain 12 on 13-hour treatment, grew vigorously. A few failed to become established, chiefly in strain 1. Data presented are based on the clones surviving and growing well on at least three of the treatments.

After the emergence of the first inflorescence above the inclosing leaf sheath on June 13, records were kept of the exsertion of inflorescences and their anthesis on each clonal division at intervals of 3-7 days for the ensuing 3 months and at longer intervals thereafter until November 17. On this date flowering had ceased on practically all plants on all treatments. Most of them in strain 12 and many in strain 5 had stopped growing, having become dormant and dying back as in previous years (6), although the plants on 15-hour treatment on this date had a greater proportion of green foliage than those on other photoperiods. Strain 1 had become dormant to a lesser degree, with 60-90% of the foliage still green on all treatments. Some tillers were still growing very slowly. Even the apical meristems of the oldest and longest culms were still alive and somewhat active in the 14- and 15-hour series.

If external conditions control the diminished activity and dormancy of this species in the autumn under greenhouse conditions, it seems apparent that growth—and especially flowering—can proceed vigorously only when natural

light intensities are above the levels common in the greenhouses at Chicago in the autumn and winter months, no matter how favorable the temperature and photoperiodic conditions. However, while seasonal light intensities at Chicago are lower than those to which these strains are subjected under natural conditions, the vigor and habits of the plants (figs. 2-10) on most treatments in the summer suggest that the intensities at Chicago in spring and summer fall within the favorable range for this species for both vegetative and reproductive behavior. The reported photoperiodic responses during these seasons, therefore, are probably not unlike those which would be obtained on similar photoperiods in the native environments of the strains, since the other critical environmental values in these experiments simulate those of the places of origin, so far as they might condition the reported responses. The results may thus be interpreted rather generally.

Results

FLOWERING SEASONS

As in previous experiments, the beginning and progress of flowering were measured by the exsertion of inflorescences from the surrounding leaf sheaths. While initiation of inflorescences probably occurs 2-4 weeks before exsertion, time and material did not permit the dissection necessary to show it. So far no other criteria have proved satisfactory for recognition of reproductive activity in these strains prior to exsertion of the first inflorescence. Anthesis followed exsertion in 7-21 days, generally after 10-14 days—except in the last few weeks of the experiment, when it was longer delayed.

The extremes and mean number of days after April 16 required by the clonal divisions which flowered to exert their

first inflorescences are given in table 1. The ascending curves in figure 1 also indicate the diversity shown among the clones within each strain on each treatment in this respect, as well as the final

TABLE 1

NUMBER OF DAYS AFTER CLONAL DIVISION AND CLIPPING ON APRIL 16, 1944, UNTIL FIRST FLOWERING ON DIFFERENT PHOTOPERIODS. AVERAGES AND EXTREMES BASED ON PLANTS WHICH FLOWERED IN TWELVE CLONES OF EACH STRAIN

STRAIN NO.	PHOTOPERIODS (HOURS)			
	13	14	15	N
12 Extremes.	91-129	64-97	71-104	71-91
Average	113	77	83	78
5 Extremes.	71-115	84-122	108-173	97-143
Average	91	104	132	124
1 Extreme.	58-84	143-194	None	157-160
Average	69	179	None	158

TABLE 2

LENGTH OF SEASON (IN DAYS) IN WHICH INFLORESCENCES WERE BEING EXERTED BY INDIVIDUAL CLONES (NOT DURATION OF EXERTION BY EACH POPULATION)

STRAIN NO.	PHOTOPERIODS (HOURS)			
	13	14	15	N
12 Extremes.	1-42	18-96	38-131	31-65
Average	9	62	76	47
5 Extremes.	18-77	1-97	1-65	1-45
Average	46	47	21	22
1 Extremes.	89-129	7-58	0	13-23
Average	109	29	0	19

percentage of flowering plants in each population. Figure 1 also summarizes data on the duration of the seasons of inflorescence exertion by the various strains on different treatments, showing the percentages of plants which were ac-

tively exerting inflorescences at various times during the season. Table 2 also shows the extremes and average duration of the periods of active exertion by individual clones. The relative production of inflorescences in each strain and treatment, based on the average of all individuals in each population, whether flowering or nonflowering, is shown in table 3. The total number of clones, rather than that of the flowering ones in each treatment, was used in the computation, since interest is centered on the vigor of flowering by each population rather than on individual clones.

TABLE 3

AVERAGE* NUMBER OF INFLORESCENCES PRODUCED PER PLANT BY POPULATIONS OF IDENTICAL CLONES IN EACH STRAIN ON EACH PHOTOPERIODIC TREATMENT

STRAIN NO.	PHOTOPERIODS (HOURS)			
	13	14	15	N
12.....	1.8	19	19	16
5.....	8	8	1.7	2.9
1.....	31	5	0	16.3

* Based on whole population, not on flowering individuals.

STRAIN 1.—The data of tables 1 and 2 and of figure 1 indicate that the selected plants of this strain from Texas are similar with respect to the criteria shown. Since they are also very similar in appearance, only one clone is illustrated (fig. 2). They are obviously intermediate or short-day plants, as shown by the absence of inflorescences on 15-hour photoperiod and the delay in exertion until September 20-23 in the N series, when inflorescences appeared on all clones. In the previous experiments (6) they first appeared on September 28 on natural photoperiod. The early and long-continued flowering in the 13-hour series in all experiments, and the long-delayed

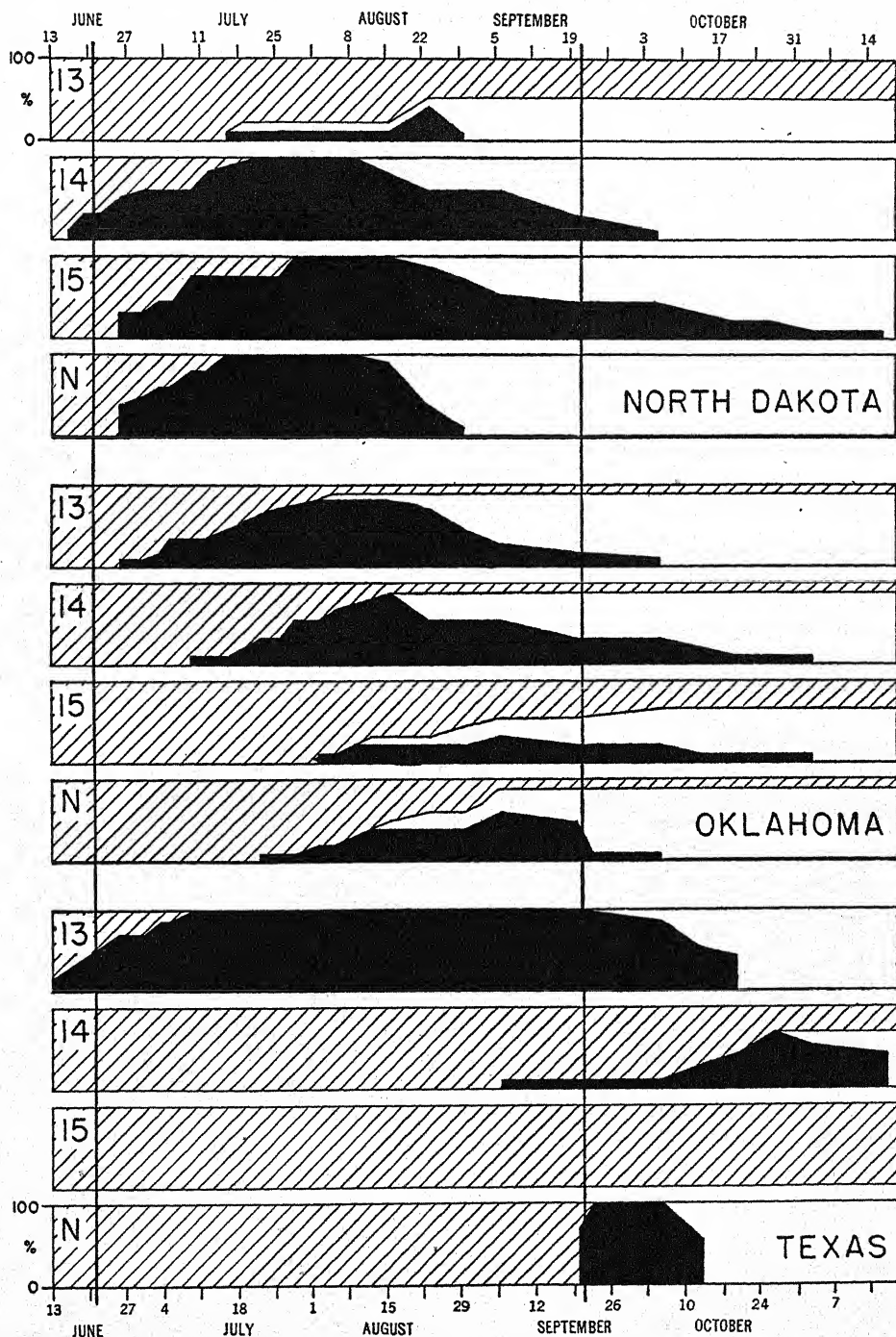


FIG. 1.—Flowering seasons at Chicago of identical clonal populations of each of three strains of side-oats grama, grown after clipping and division on April 16, 1944, on photoperiods of 13, 14, and 15 hours, and natural daylength (N). On any particular date, the value shown by the shaded area is the percentage of plants actively exerting inflorescences; hatched area, percentage which had not flowered; and white area, percentage which had ceased flowering.

and limited flowering on 14-hour photoperiod, strikingly confirm this conclusion. All the data suggest that an upper critical photoperiod for rapid and vigorous flowering lies slightly below 14 hours. Thus the initiation of the first inflorescences to be exerted in the N series must have occurred near the last week in August, when the effective photoperiod at Chicago first falls to this level after the summer solstice (the period between sunrise and sunset on August 31 is 13 hours and 12 minutes). The similar decline to effective photoperiods of approximately 14 hours in southern Texas occurs in early August. During the early part (March, April) of the average growing season at San Antonio, natural photoperiods are also below this level. Information has not been obtained on the time of initiation of growth or of inflorescences by this strain in that area. Seed is ripe by September 15 at San Antonio. Under the warm temperature conditions, inflorescences bearing it might well ripen within 6 weeks after an indicated initiation in late July or early August.

The intermediate- or short-day status of these clones has not been determined, even though exertion of new inflorescences ceased on October 13 in the N series. These last inflorescences to appear must have been initiated on effective photoperiods of $12\frac{1}{2}$ –13 hours or more. The cessation of flowering, however, was probably not due to the decrease of the photoperiod to an unfavorable length for initiation, since previous experiments have shown that individuals in this strain will grow and *some* (but not all) will flower on daily photoperiods of 9 hours in the summer months at Chicago, although much less vigorously than on 13-hour photoperiods. A 9-hour photoperiod is below the level to which they are naturally subjected, even when

dormant. Rather, the short flowering season and cessation of flowering in the N series occurred as a consequence of the apparently nearly simultaneous transformation of *all* apical meristems on tillers of any considerable size into inflorescence primordia in response to the decrease of daily photoperiods to a certain length in August and September. Dormant buds and small tillers which might have prolonged the reproductive activity had either not attained a size favorable for initiation or were inhibited in growth as a consequence of the flowering of the older culms.

Inflorescences in the N series thus appeared simultaneously on culms which began to elongate soon after transplanting in April and on the larger of those which developed later during the summer. As a consequence there was a wide gradation in mature culm length and in the number of elongated internodes per flowering culm at anthesis, the longest and oldest ranging from 80 to 90 cm. long with eight to eleven elongated internodes, while those just beginning to elongate at the time of floral initiation attained lengths of 50–60 cm. with five or six elongated internodes. It is obvious that internodal elongation may precede and is neither a sign nor an effect of reproductive activity in this strain. Stems as long as 100 cm., with thirteen elongated internodes, had been formed in the completely vegetative 15-hour series by November 17, and their apical meristems were still active. The few inflorescences produced in the 14-hour series, chiefly exerted in October, were all borne on long tillers with numerous internodes. In the 13-hour series, however, the photoperiodic stimulus to floral initiation became effective early in the development of each successively formed tiller, so that, once initiated, flowering in most

clones continued until late September or October, the last inflorescences appearing on October 20. Culms ranged 35–80 cm. in length and possessed three to five elongated internodes. They were almost twice as numerous as in the N series (table 3) but on the average were smaller in size. The great delay in flowering in the N series allowed the older culms to continue vegetative growth for a long

since small young tillers, showing some internodal elongation, were present on November 17. They were apparently vegetative, although they were not dissected.

The variation in time of first exertion and in duration of flowering by the various clones in the 13-hour series (tables 1, 2) was probably due largely to accidental differences in rate of establish-



FIG. 2.—Divisions of one clone of strain 1 (Texas), grown after April 16, 1944, on photoperiods of (left to right): Chicago natural daylength, 13, 14, and 15 hours. Photographed August 26, 1944. Division on natural photoperiod flowered vigorously from September 20 to October 13; division on 13-hour photoperiod exerted inflorescences from June 26 to October 6; the others failed to flower.

time. Consequently, fewer tillers were produced than in the 13-hour series, where vegetative growth of each tiller was soon brought to a close by the initiation of an inflorescence primordium, and additional tillers were continuously forming and developing.

The cessation of inflorescence exertion in the 13-hour series on October 20 may have been partly a consequence of decreased light intensity or other factors,

ment after transplanting, rather than to genetic differences affecting the rapidity of initiation on the 13-hour photoperiod.

In summary, this population of strain 1 consists of intermediate- or short-day plants, somewhat similar in habit and response within each treatment of this experiment, with an upper critical photoperiod of approximately 14 hours. Any intermediate-day plants also have a lower critical photoperiod between 9 and 13



FIGS. 3, 4.—Divisions of two clones of strain 12 (North Dakota), grown after April 16, 1944, on photo-periods of (left to right): Chicago natural daylength, 13, 14, and 15 hours. Photographed August 26, 1944. Clone in fig. 3 (above) represents extreme in limitation of vegetative growth on 13-hour photoperiod. Most clones of this strain more closely resembled the one shown in fig. 4 (below) in both vegetative and reproductive habits, although its leaves in the 13-hour series are somewhat more erect and longer than the average.

hours. In their native environment in southern Texas they would seem to be adjusted by photoperiodic requirements to a long season of vigorous vegetative growth during the long days of spring and early summer, but they are delayed in flowering until late summer. Seed is ripe, however, long before any danger of frost in November. No data are available to the writer on the growth habits of this

toperiod, unless following as a consequence of the reproductive activity induced in part by this factor. By reason of this type of adjustment to the temperature and photoperiodic conditions in southern Texas, similar strains, when planted in more northern latitudes, continue vigorous vegetative growth on the long summer days (more than 14 hours) until August or September, may fail to



FIG. 5.—Divisions of one clone of strain 12 (North Dakota), grown after April 16, 1944, on photoperiods of (left to right): Chicago natural daylength, 13, 14, and 15 hours. Photographed August 26, 1944. This clone showed the maximum flowering response in this strain on 13-hour photoperiod (and approximately maximum vegetative growth on this treatment).

strain in the autumn months in southern Texas. The experiments indicate that photoperiod would not be a limiting factor of significance for vegetative growth in the autumn, or even winter months, since plants remain partly green and grow somewhat in the even shorter days of winter at Chicago, and on experimental 9-hour photoperiods in summer. The onset of dormancy or limited growth of this strain in Texas in autumn is thus probably not related to decreasing pho-

ripen seed before autumn frosts, and are often winter-killed (7), in contrast with strains (such as strain 12) well adjusted photoperiodically to the northern conditions.

STRAIN 12.—The individuals of this strain from North Dakota showed greater variability than did those of strain 1, although not so much as did those of strain 5, both in flowering responses (tables 1, 2, 3; fig. 1) and in vegetative habit (figs. 3-5). Note especially the

variation shown in the 13-hour series. However, it is obvious from the data and from their appearance that all the selected individuals are intermediate- or long-day plants, with a short critical photoperiod for both vigorous growth and flowering between 13 and 14 hours. While more than half the clones were eventually able to initiate and exert one or more inflorescences in the 13-hour series, the plants were small and the inflorescences few, weak, sterile, and exerted later in each clone, in contrast with the other three series (figs. 3-5; table 2). This critical photoperiod is approximately of the same length as the minimum natural photoperiod to which this strain is exposed in its growing season in North Dakota.

It is also of interest that the last inflorescences were exerted in the N series on August 30. These were probably initiated in late July or early August on decreasing photoperiods considerably in excess of 14 hours. Since flowering was as vigorous on 14- and 15-hour photoperiods as in the N series (table 3), and was much longer continued (fig. 1; table 2), the reason for the cessation of exertion in the N series on August 30 is not entirely clear. It is possible that inflorescences were initiated in August in some plants which were not exerted because of the effect of photoperiods under 14 hours in limiting internodal elongation. Effective photoperiods decrease to this value in late August and early September at Chicago. Several of the clonal divisions in the N series became semidormant at this time, while others were still producing a few short tillers. In the former, dormancy may naturally follow as a consequence of a vigorous reproductive phase, irrespective of photoperiodic conditions, since the divisions of some of these clones in the 14- and 15-hour series

ceased activity at about the same time (fig. 1). It is also possible that other external or internal factors may have been responsible. This is suggested by data on inflorescence number, culm length, and internode number. These values were approximately the same in the 14-, 15-hour, and N series for those culms elongating in June, July, and August. These are the culms visible in figures 3-5. They ranged from 55 to 80 cm. long, averaging about 70 cm., with four or five elongated internodes in all cases. The few flowering culms in the 13-hour series were much shorter (35-60 cm.), and some had only three elongated internodes. Those culms in the 14- and 15-hour series which elongated in September and October were shorter than those appearing earlier, ranging from 40 to 50 cm., but with the same internode numbers of four or five. The inflorescences were also smaller. Thus, while there was still a stimulation to activity in the autumn in these two series in contrast to the N series, in spite of similar vigorous flowering previously, it was not so effective as it had been, as shown by shorter internodes, smaller inflorescences, and a decrease in the rate of production of new inflorescences. While continued activity was probably stimulated chiefly by the long photoperiods, it is not clear what factors were responsible for the diminution of flowering in these two series. It does seem possible, however, that, in addition to these unknown factors, decreasing photoperiod did play a part in the onset of dormancy in the N series, which led to a practical cessation of both vegetative and reproductive activity in all clones, while some of those on longer photoperiods were still active. Plants of this strain, however, did grow very weakly on a 9-hour photoperiod in the previous experiments. It is possible that the effects of decreasing

short photoperiods are somewhat different from those of constant ones.

All these clones in the N series were able to initiate and exert inflorescences on the longest days of June and July (effective photoperiods of 16 hours or more), although exertion began slightly earlier in the 14-hour series than on natural or 15-hour photoperiods. The divisions of most individual clones, however, began flowering less than 10 days apart in these three series. Since numerous plants flowered on 20-hour photoperiods in previous experiments, it would seem that, if any of the individuals in the present experiment are intermediate- rather than long-day plants, their upper critical photoperiod is probably above 16 hours and thus of little practical significance in regulating development in their native environment.

The data indicate that these plants are well adjusted to the range of photoperiods in the frost-free growing season in North Dakota. After resuming growth in late April or early May,² they can initiate inflorescences as soon as they attain sufficient size. They may continue to flower—so far as photoperiodic restriction is concerned—until such time as the inhibiting effect of photoperiods decreasing to a certain level, and other factors, lead to the limited growth and onset of dormancy in late August and early September, which enables them to escape injury from the first autumn frosts of mid- or late September. According to Mr. ROGLER, seed is usually ripe at Mandan by August 20, and the plants are completely dormant by September 15, 10 days before the average date of the first killing frost. In so far as photoperiodic responses control the developmental

cycle, these plants are thus well adjusted for seed production to the long photoperiods of the relatively short frost-free season of their native environment. They might produce more forage if they had an upper critical photoperiod of such length as to cause a delay in flowering until later in the summer, but they would then be less well adjusted to escape early frosts. ROGLER (7) classes side-oats grama as a "warm-temperature" grass in North Dakota, since it is dormant in the cool spring and fall months, during which "cool-temperature" grasses show active growth. This is in accord with its probable evolutionary history, with origin in low latitudes (6). The strain in North Dakota has evolved to become well adjusted photoperiodically to that latitude, while apparently retaining the same minimum temperature requirements for growth shown by more southern strains (7). It can, however, well endure winter temperature conditions unfavorable for southern strains.

STRAIN 5.—This strain showed considerable variability in vegetative habit and in flowering among the various clones (figs. 6-10). In general, the data confirm the conclusions based on non-identical populations in the previous experiments—that this strain consists of both intermediate- and long-day plants. Flowering was less vigorous than in strains 1 and 12, although the plants grew well vegetatively on all treatments. For the strain as a whole the data do not show a well-defined uniform critical photoperiod, and the range of photoperiodic conditions of the experiment apparently controlled development less decisively than in the other two strains.

All clones except three eventually flowered on all four treatments. For the majority the long photoperiods in the 15-hour and N series apparently caused

² Data on growth in North Dakota from personal correspondence with Mr. GEO. A. ROGLER, Northern Great Plains Field Station, Mandan, N.D.

some delay in the exertion of inflorescences, which were also few in number, in contrast with the development in the 13- and 14-hour series (figs. 1, 6, 7; tables 1, 3). Apparently correlated with the delay in flowering was a greater number of internodes per flowering culm on the longer photoperiods (N and 15-hour). Three clones were delayed in flowering in the N series until the first week in September. All clones except one flowered first in the 13- or 14-hour series. Three clones failed to flower on 15-hour photoperiod. One (fig. 9) of these three flowered on the other three treatments, another only in the 13-hour series (fig. 10), and the third in the 14-hour and N series. The failure of the first of the three to flower in the 15-hour series was most probably due to unfavorable photoperiod, since it flowered vigorously in both 13- and 14-hour series, and eventually in the N series. This clone is thus an intermediate-day plant whose upper critical photoperiod lies between 14 and 15 hours. The flowering responses of the clone which flowered weakly only on 13-hour photoperiod (fig. 10) was probably limited by genetic conditions rather than external ones, although a short-day plant with a critical photoperiod between 13 and 14 hours might be able to ripen seed in the native environment of this strain. Little emphasis should be placed on this clone or on the one which flowered only on 14-hour and natural photoperiods. Flowering in the latter was relatively weak in both treatments. The four inflorescences appearing in the N series were exerted between July 22 and August 16, and thus they must have been initiated on photoperiods of 15 hours or more. It is unlikely that failure of this clone to flower in the 15- and 13-hour series was due to unfavorable photoperiods.

Average number of inflorescences per plant was the same in the 13- and 14-hour series (table 3). A higher percentage of the clones exerted them simultaneously in these two series than in the other two (fig. 1), although the flowering seasons were more prolonged in the 14- and 15-hour series (to November 3) than on natural or 13-hour photoperiods (to October 6). It is not clear why exertion ceased in the 13-hour series on this date. A few vegetative tillers were still being produced and growing, although with limited internodal elongation. By November 17 this series was mostly dormant, in contrast with continuing limited growth of some clones in the 14- and 15-hour series. The relatively vigorous early flowering may have hastened the onset of dormancy in the 13-hour series, especially if some of these clones have a lower critical photoperiod for vigorous growth and flowering near but somewhat below 13 hours. In previous experiments there was no flowering on 9-hour photoperiods, and vegetative growth was weak in comparison with the 13- and 16-hour series, although less so than in strain 12.

If flowering ceased in the N series in the present experiment partially as a consequence of unfavorable photoperiods, the last inflorescences to appear were probably initiated in early September on photoperiods about $13\frac{1}{2}$ hours long. However, some clones ceased flowering in mid-August. Their inflorescences must have been formed during the long days of July. Since these clones also flowered well on 13-hour photoperiod, their cessation of flowering in August in the N series does not suggest that it was due to decreasing photoperiods. In previous experiments some plants of this strain flowered on 16- and 20-hour photoperiods.

Most clones were in flower simultane-

ously, at least for a time, in the 13- and 14-hour treatments, in comparison with their seeming differentiation into "early" and "late" individuals in the N and 15-hour series. This resulted from the considerably greater delay in flowering of some clones in the last two treatments and suggests that still longer photoperiods might have resulted in a differentiation into flowering and nonflowering individuals. If this should prove to be the case, the population would thus contain some individuals with an upper critical photoperiod, in addition to the one clone previously mentioned, although the length of this photoperiod might vary from plant to plant. Since no plants have flowered on 9-hour photoperiods, it seems reasonable to assume that all the individuals have a lower critical photoperiod between 9 and 13 hours, and thus none would be short-day plants. As in strain 12, this short critical photoperiod might lie near the lower limit of the photoperiodic range in which these plants are active in their native environment. Those plants with an additional upper critical photoperiod would be intermediate-day plants, while those able to flower on 20 hours are probably long-day plants. So far as photoperiodic conditions control their development, the latter should be able to flower at any time during the relatively long growing season in Oklahoma when they have attained sufficient size, and they are thus probably the "early" ones; while those with an upper critical photoperiod near 15 hours, or which are delayed in flowering on a 15-hour as compared with a 14-hour photoperiod, might well be delayed until later in the season. This strain begins to grow at Manhattan, Kansas, about April 20, usually exserts its first inflorescences about July 1, and reaches its peak of flowering about July 12.³ In the Soil

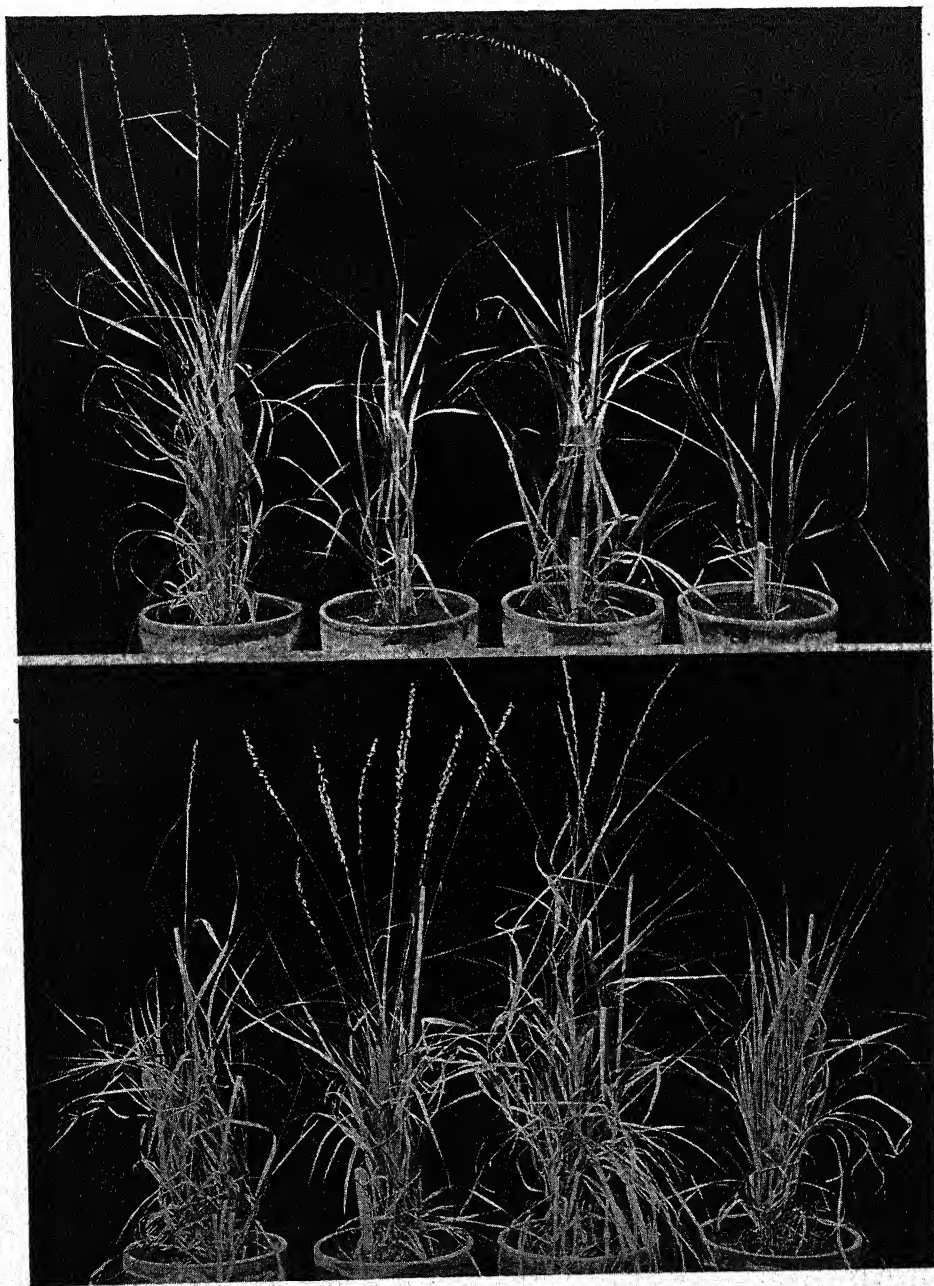
Conservation Service Nursery at Manhattan, where this has proved to be a highly desirable strain, a crop of seed is usually harvested in early August. If conditions remain favorable a second crop is produced in September, which is harvested in October. It would be possible, so far as photoperiodic requirements indicated by the present experiments are concerned, for many plants to contribute to both seed crops, while others might be so delayed in flowering on the long days of midsummer as to fail to reach maturity before early August but could produce seed for the October harvest. The latter type of plant, if it occurs, would probably produce the greatest amount of forage under field conditions, especially in wet years, since its vegetative growth would not be limited as a consequence of the early onset of reproductive activity. Under natural conditions this variability in photoperiodic requirement should lead to a long flowering season, with a greater likelihood of some seed production each year than if all plants matured at nearly the same time, as they apparently do in strains 1 and 12.

The available data do not show whether the onset of dormancy in this strain is hastened by decreasing photoperiods, although this is possible, or whether vegetative growth is much limited by this factor during the late part of the growing season in the Oklahoma environment. At Manhattan, Kansas, it is dormant by October 10, approximately the average date of the first killing frost. Some plants in the N series were still growing slightly and their green foliage ranged 30-60% on November 17, when the natural photoperiod at Chicago had decreased to a level below that at which this strain is

³ Personal correspondence with Mr. D. R. CORNELIUS, Soil Conservation Service Nursery, Manhattan, Kansas.



FIGS. 6, 7.—Divisions of two clones of strain 5 (Oklahoma), grown after April 16, 1944, on photoperiods of (left to right): Chicago natural daylength, 13, 14, and 15 hours. Photographed August 27, 1944. Note contrasts in vegetative habit among clones shown in figs. 6–10. Clone in fig. 6 (above) is a long-day plant, although it flowered first and more vigorously on 13- and 14-hour photoperiods. Clone in fig. 7 (below) flowered on 15-hour photoperiod in September.



FIGS. 8, 9.—Divisions of two clones of strain 5 (Oklahoma), grown after April 16, 1944, on photoperiods of (left to right): Chicago natural daylength, 13, 14, and 15 hours. Photographed August 27, 1944. Clone in fig. 8 (above) flowered on 15-hour photoperiod in October. Clone in fig. 9 (below) is an intermediate-day plant, with upper critical photoperiod between 14 and 15 hours, and was considerably delayed in flowering on natural and 14-hour photoperiods in comparison with the 13-hour division.

normally subjected to frost in Oklahoma. In contrast, however, 50–80% of the foliage in the 15-hour series was still green on this date, with slightly less amounts in the 14-hour series. It is possible that the greater longevity of foliage in the latter two series is related to the artificial light which they were receiving when natural light intensities had decreased to low values, and not to the effects of longer photoperiods. Both factors may be responsible, since the 13-hour

in contrast with development in the 13- and 14-hour series—are probably intermediate-day plants. Other individuals, able to flower on 20-hour photoperiods, are probably long-day plants, although the data of 3 years indicate some delay in exsertion of inflorescences on such long photoperiods in contrast with 13-hour treatments. This variability in photoperiodic response should make it possible to acclimatize certain selections from this strain to areas considerably north or



FIG. 10.—Divisions of one clone of strain 5 (Oklahoma), grown after April 16, 1944, on photoperiods of (left to right): Chicago natural daylength, 13, 14, and 15 hours. Photographed August 27, 1944. This clone is strongly vegetative on all treatments and is not classified photoperiodically.

series showed death of foliage comparable with the N series.

In summary, this strain is highly variable in both vegetative (figs. 6–10) and reproductive habits. All individuals tested apparently have a lower critical photoperiod between 9 and 13 hours, while one of the experimental plants likewise has an upper critical photoperiod between 14 and 15 hours. This clone, and several which were considerably delayed in flowering on 15-hour photoperiod and on the long days of summer at Chicago—

south of its native latitude, so far as photoperiodic limitations are concerned.

VEGETATIVE GROWTH

The present experiment adds information to one point which merits attention. In the previous experiments it was shown that vegetative growth of strain 12 on 13-hour photoperiod was very limited in contrast with behavior in the 16- and 20-hour series. Figures 3–5 indicate that the critical photoperiod for such severe limitation falls between 13 and 14 hours.

This is the more striking if one bears in mind that these series differed by only one hour of artificial light of low intensity, each receiving the same 9 hours of natural daylight. While such sharp limitation is often found in long-day plants, the contrast is very great in this strain. Whatever the mechanism physiologically, for vigorous growth this strain apparently requires a photoperiod as long as the minimum to which it is exposed in its natural growing season.

Such sharp differences in vegetative growth over a narrow range of photoperiods have not been indicated in strains 1 and 5. The differences among treatments in the present experiment are related primarily to the effect on reproductive activity of the different photoperiods, with correlated gross effects on vegetative growth and habit, and not obviously to an influence of photoperiod expressed directly on leaf size, internodal elongation, and other vegetative characters. While a critical photoperiod for flowering in strain 5, and for some plants of strain 1, lies between 9 and 13 hours, differences in vegetative habit between these two treatments in each strain are less than between the 13- and 14-hour series of strain 12. A sharp photoperiodic limit below which vegetative growth is severely limited should thus not be expected in strains 1 and 5, at least between 9 and 13 hours of light per day. This is in general accord with the responses of many short-day plants adjusted to the photoperiodic conditions of low latitudes, such as strain 1. Thus, while strain 5 contains intermediate- and long-day individuals so far as flowering responses are concerned, in some respects its vegetative habits in response to a range of photoperiods are more like strain 1 than 12. It is thus suggested even more strongly than its latitudinal position would indi-

cate that strain 5 does represent a transitional condition between strains 1 and 12.

Discussion

Many points developed at length in the preceding paper (6) will not be repeated here, and reference is made to the "Discussion" in it for conclusions additionally confirmed by the new data. The present experiment on clonal divisions of the three strains does confirm most of the conclusions previously based on non-identical populations, as to the nature of the photoperiodic responses, and indicates more precisely the length of critical photoperiods for selected individuals in each strain, the diversity within strains, and the practical importance of photoperiod in the seasonal and latitudinal adjustment of the strains.

The selected individuals of strain 1 (Texas) showed less diversity than did those in strains 5 (Oklahoma) and 12 (North Dakota) in all respects. Strain 5 is more variable than strain 12. FULTS's data (3) suggest that strain 1 lies east and south of the present center of greatest genetic and cytological diversity of the species in the United States, whereas strain 5 is native in this center, and strain 12 is considerably north of it.

All the tested individuals of strain 1 from southern Texas are intermediate- or short-day plants, with an upper critical photoperiod for rapid and vigorous flowering between 13 and 14 hours. Some clones flowered weakly after long delay on 14-hour photoperiod. The selections included one plant which had previously flowered weakly on 16-hour photoperiod after 8 months of growth but did not flower on 15-hour photoperiod in this experiment. While the strain may include other individuals eventually able to flower on such long photoperiods, from the standpoint of regional adaptation they

apparently are all short-day plants which would probably be unable to ripen seed if planted very far north of their native latitude, although they are able to produce luxuriant vegetative growth in their first year on the longer days of northern latitudes. They suggest that long-day individuals or intermediate-day ones with a longer critical photoperiod might evolve from this strain in a slow northward migration. In its native environment, photoperiod undoubtedly exerts some control over the developmental cycle by prolonging vegetative activity and causing some delay in the initiation of inflorescences, but probably it does not directly influence dormancy or limit growth in the autumn. This strain is still non-rhizomatous under the experimental conditions, in contrast with strains 5 and 12.

In the North Dakota strain the new data strikingly confirm a long-day status. The critical photoperiod lies between 13 and 14 hours, below which vegetative growth is very much limited, with little internodal elongation, although some clones flowered weakly. Inflorescence initiation probably occurs rapidly on photoperiods above this level in June and July in North Dakota, while the onset of dormancy in late August and September coincides with the decrease of photoperiod below the critical. Thus this strain is active in North Dakota for about a 4-month period and is completely dormant before danger of frost. If planted too far south of its native latitude, in areas where daylengths exceed its critical for only the few weeks centering on the summer solstice, it would show very limited growth and flowering in comparison with more southern strains.

Strain 5 from central Oklahoma shows much more variability and consists of both intermediate- and long-day plants, with a lower critical photoperiod below

13 hours. Vegetative growth is vigorous on photoperiods of 13 hours or more. The intermediate-day plants probably vary in the value of the upper critical photoperiod. In one individual it lies between 14 and 15 hours. Such variability should lead to a long flowering season, and, so far as photoperiodic conditions are concerned, to relatively easy acclimatization of selections from this strain to areas north or south of its native latitude.

The short-day condition of strain 1 was probably the original photoperiodic response in the species, as pointed out previously (6), since the species undoubtedly spread to the north after an origin in low latitudes—where this response is now typical. This would have involved the evolution of the intermediate- and long-day responses as the migration occurred. If crosses between the different strains are possible, a photoperiodic study of the parental, F_1 , and F_2 generations might yield data on the possible evolutionary mechanism of such adjustment, as suggested by the recent work of GOODWIN (4). In conjunction with fundamental studies on the physiology of photoperiodism in the various strains, including vegetative as well as flowering responses, it might be possible to ascertain whether the differences between short- and long-day plants are absolutely qualitative or are primarily quantitative in nature. The existence of both types in one species, linked by transitional ones (such as occur in strain 5), suggests that the qualitative differences between the types are the visible expression of only quantitative differences in the physiological mechanism which induces flowering.

Summary

1. Clonal divisions of twelve selected individuals of each of three strains of side-oats grama were grown from April

16 to November 17, 1944, on Chicago natural daylength and on photoperiods of 13, 14, and 15 (or more) hours. The three strains from Texas (San Antonio), Oklahoma (El Reno), and North Dakota (Cannonball) represent the extremes and approximate means of latitudinal origin and range of response of twelve strains previously grown over a 2-year period on 9-, 13-, 16-, 20-hour, and natural photoperiods. The selected individuals sampled the diversity of each strain.

2. All clones from Texas flowered rapidly and persistently on 13-hour photoperiod, and in late September on natural photoperiod. Some flowered weakly in September and October in the 14-hour series. None flowered in the 15-hour series. Vegetative growth was vigorous on all treatments. These clones are intermediate- or short-day plants with an upper critical photoperiod for rapid and vigorous flowering between 13 and 14 hours. All clones from North Dakota grew and flowered vigorously on natural, 14-, and 15-hour photoperiods. On 13-hour photoperiod growth was very limited, although some clones flowered weakly. These clones are long-day plants with a critical photoperiod for vigorous growth and flowering between 13 and 14 hours. Most clones from Oklahoma flowered equally well and fairly rapidly on 13- and 14-hour photoperiods. Most of them eventually flowered on natural pho-

toperiod, but were delayed, in contrast to the 13- and 14-hour series. Most of them either failed to flower or were delayed in flowering on 15-hour photoperiod. These data and previous observations indicate that this strain includes both intermediate- and long-day plants, both types having a short critical photoperiod between 9 and 13 hours. One of the former has an upper critical photoperiod between 14 and 15 hours.

3. Such strong photoperiodic differentiation and adjustment have seldom been reported within a single native species. The series of photoperiodic types within it raises interesting questions as to the evolution and nature of the responsible genetic and physiological mechanisms, since the species probably originated in low latitudes, where the short-day response is typical, with subsequent adjustment to the longer days encountered in the northward extension of its range. Other implications of the findings discussed in this or the preceding paper (6) concern some of the causes of the morphological diversity among the latitudinal strains when grown together, as reported by other workers, and the bearing of photoperiodic adjustment upon possible acclimatization of strains to latitudes lower or higher than their native ones.

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THE GAMETOPHYTE OF DIPTERIS CONJUGATA

ALMA G. STOKEY

Introduction

The genus *Dipteris* has been a taxonomic problem ever since REINWARDT established it in 1824 with the species *D. conjugata*, which had previously been placed in the genus *Polypodium*. Several of the later pteridologists of the nineteenth century returned *D. conjugata* (and its related species as they were described) to the genus *Polypodium*, on account of the naked sorus. DIELS (8) placed the genus *Dipteris* in the Aspidaceae, stating that it is an isolated type but in vegetative characters has more in common with the Aspidaceae than with other groups. CHRISTENSEN (5) in 1906 gave it the same position as DIELS. SEWARD and DALE (10), however, in 1901 had approached the problem from the morphological and paleobotanical side and met the difficulties by the establishment of a new family, Dipteridineae (Dipteridaceae). Since then the sorus of three species has been investigated by ARMOUR (1), who considered *D. conjugata* the most advanced of the three in soral characters. The ontogeny of the stele in two species, including *D. conjugata*, was investigated by DE BRUYN (7). BOWER (2) in 1915 discussed *Dipteris* and its probable derivatives in connection with the study of *Cheiropleuria bicuspis*, and again (3) in 1926, with reference to its phylogeny as indicated by anatomy, leaf architecture, sorus and sporangium—emphasizing (as did SEWARD and DALE) its similarities to *Gleichenia* and *Matonia*. BOWER accepted the family Dipteridaceae, and this position has been taken in several recent works treating of the phylogeny of ferns; although as late as 1938 Christensen

(6) retained *Dipteris* in the Polypodiaceae, assigning it to the subfamily Dipteridoideae between the subfamilies Dryopteridoideae and Polypodioideae. It has been pointed out, in considering the position of *Dipteris*, that a weak point has been our lack of knowledge of the structure and development of the gametophyte. We have had only the figure shown by BOWER (3, p. 318) of a mature gametophyte collected by Dr. LANG on Mount Ophir, Malay Peninsula.

Material and methods

The spores of *Dipteris conjugata* Reinw. were obtained from fertile leaves collected by the writer from plants growing in the Botanical Garden at Tjibodas, Java. Although plants were seen growing wild in the mountains, they were much less accessible. The plants in the garden were growing in large clumps and were vigorous, healthy, and fruiting abundantly. The first collection and cultures were made in 1931, but the cultures did not live long enough to develop late stages of the gametophyte. On a second visit to Tjibodas in 1937, collections of spores were again made and cultures started. The spores were planted within a few hours after they were collected; they lost their viability quickly and would not germinate when more than a few days old. The early stages were studied in the Treub Laboratorium, Buitenzorg; then the cultures were brought to the United States and work continued at Mount Holyoke College and the Marine Biological Laboratory, Woods Hole, Massachusetts. The gametophytes are still in culture. Germination stages were obtained on distilled water

and on peat, and the later stages on peat by methods described in a previous paper (11). Fresh gametophytes were used for the study of germination stages, habit of growth of later stages, distribution and external aspects of sex organs, and habit of growth of apogamous plants. Sections were used for internal structure; for killing and fixing, a weak to medium chromo-acetic acid solution and a modified Navashin's solution were used.

Observations

SPORE GERMINATION

The spores of *D. conjugata* are bilateral, varying in size from 18×35 to $21 \times 44 \mu$ (fig. 1). SEWARD and DALE described the spores of *Dipteris* as bilateral, but CHRISTENSEN (6) has described them as tetrahedral. The spores are pale, almost colorless, with a smooth thin coat without perispore, and appear whitish in the mass. A considerable number of sterile spores without granular contents appeared in the collections. The sporangia do not discharge effectively, and some spores are left in the sporangium. The spores are less powdery than in the case of most ferns and tend to stick together, making it unusually difficult to get well-distributed prothalli in cultures; accordingly, the spores germinate in groups, and this tends to make the gametophytes irregular in form. There were often detached sporangia in the cultures, and the spores which remained inside germinated readily. The germination period was about 2 weeks, which is rather slow. The first division of the spore was usually perpendicular to the long axis, and one of the two cells usually produced a rhizoid (figs. 2, 5, 6), although a rhizoid arose as a result of the first division in some cases (figs. 3, 4, 8). In most cases a plate was formed, or less frequently a

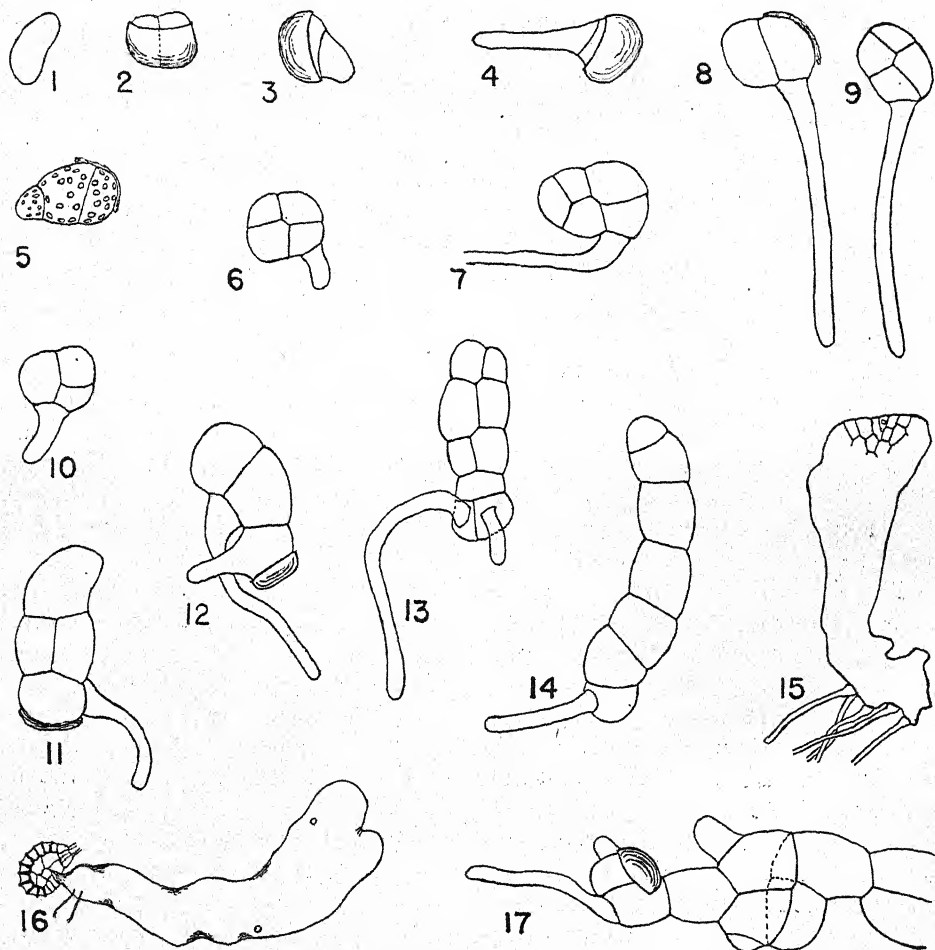
more or less irregular mass, with great variation in germination stages (figs. 7, 9-13). In this material germination seldom resulted in a filament, and if one was formed—as in the case of germination in a sporangium (fig. 14)—the tendency to form elongated cells was weak or lacking. When a spore germinated in the sporangium the prothallus usually broadened by lengthwise divisions in the filament as soon as the anterior portion became free (fig. 16). Considerable enlargement of the cells of the plate usually preceded elongation of the thallus. In the early stages the gametophytes grew very slowly and in 4 weeks had formed not more than four to six cells and a rhizoid.

GAMETOPHYTES

After the early stages, the development of the young gametophyte is that of the usual type found in leptosporangiate ferns, with growth from a single apical initial (fig. 15) followed by growth from a group of initials and formation of a heart-shaped prothallus with midrib. The form of the young regenerated gametophytes in figure 18 gives the type of those arising from spores. If conditions are unfavorable and the thallus is crowded, it may assume a ribbon-like form with a cordate apex (fig. 16). The rhizoids are pale brown in color and are usually limited to the region of the midrib, but in old gametophytes the rhizoid region is not always continuous along the rib. Many chloroplasts were found in young rhizoids, particularly in the case of young gametophytes. Under some conditions there is considerable irregularity in the development of the plate and the region at the base of the thallus (fig. 17). In cultures 4-5 months old there were many small gametophytes, consisting of relatively few cells, frequently with irregular bases, suggesting an asymmetri-

cal type of germination as a mass. Even in the irregular gametophytes there were few which had filamentous portions. The cultures were in transit from Java to the United States during the early stages of development, and although every effort

ever, the usual effect of unfavorable conditions is to cause them to become filamentous, but this rarely happened in *D. conjugata*. Greater irregularities were found in this species than in the various others subjected to the same conditions.



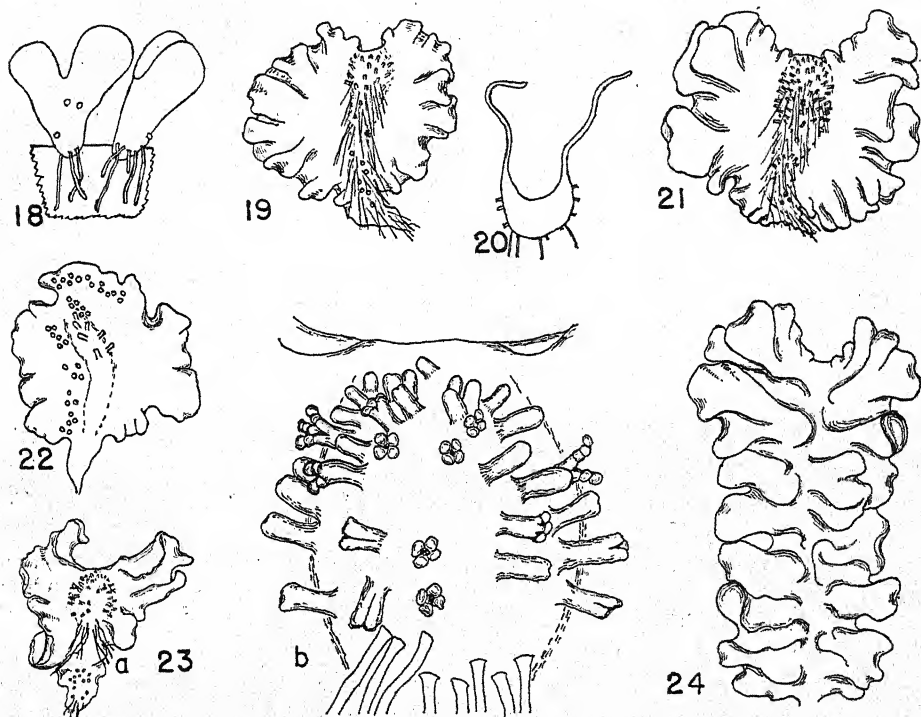
FIGS. 1-17.—Fig. 1, spore. Figs. 2-14, early stages of gametophyte. Fig. 15, gametophyte at apical stage. Fig. 16, gametophyte which has germinated in sporangium. Fig. 17, irregular base of young gametophyte.

was made to keep them in suitable light with little change of direction, the exigencies of travel may be responsible for some of the irregularities. In the case of the gametophytes of most ferns, how-

The mature gametophytes have a heavy midrib and ruffled wings which may be more or less lobed (figs. 19, 21, 24). The top of the rib of an old thallus may be entirely concealed by the wings,

and the cross-section of the thallus may be like that in figure 20. The older parts of the thallus usually died away at a relatively early age, so that the gametophytes in these cultures never attained the great length of thallus which may be found in old gametophytes of *Osmunda*. The ruffled wings on the relatively short

in several other species in culture. They show the same type of ruffled wings and heavy midrib, both in surface view and in section. The tendency of the midrib of old gametophytes to branch, which is not unusual in the Osmundaceae, Gleicheniaceae, Cyatheaceae, and Dicksoniaceae, was almost lacking in these cul-



FIGS. 18-24.—Fig. 18, portion of old thallus with two regenerated gametophytes bearing antheridia. Figs. 19, 21, mature gametophytes bearing antheridia and archegonia. Fig. 20, cross-section of old thallus with archegonia and rhizoids. Fig. 22, gametophyte which bore first antheridia, then archegonia, then antheridia. Fig. 23: *a*, mature gametophyte with antheridia and archegonia; *b*, cushion of same thallus with archegonia and rhizoids. Fig. 24, old gametophyte about 7 mm. long, dorsal view.

thallus often suggest a head of curly lettuce. In a few gametophytes a certain amount of starch was found stored in the midrib, as was reported for *Marattia sambucina* (12). In appearance the mature gametophytes resemble those of *Gleichenia*, as shown by CAMPBELL (4) in his figures of *G. pectinata*, *G. laevigata*, and *G. dichotoma*, and as found by the writer

tures; only a few gametophytes gave even a suggestion of a forking of the midrib, and in no case was it long-continued. No hairs of any type were found on the gametophyte of *D. conjugata*, even on those old enough to have more than 100 archegonia. Aging gametophytes may show great vigor in the production of regenerated branches (fig. 18). Cultures

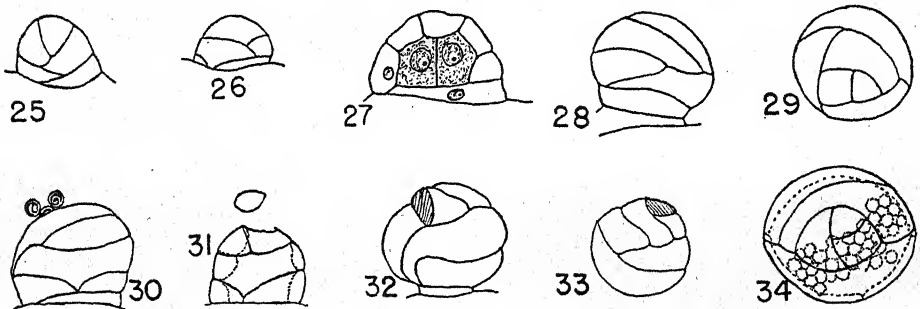
which were started in July, 1937, are still flourishing after five or six transplantings—made possible in part by the abundant production of regenerated branches.

REPRODUCTIVE STRUCTURES

The mature gametophyte of *D. conjugata* is monoecious, but the production of antheridia is initiated before that of archegonia (figs. 18, 19, 21–23). If conditions cause a check in the formation of archegonia there may be a vigorous production of antheridia, not only on the wings but also on the cushion behind the

phytes are likely to have antheridia on the margin as well as on the dorsal side.

The antheridium is of the primitive and complex type found in *Osmunda* and *Gleichenia*. The wall consists typically of five or six cells, but there is considerable variation which seems to be related to the size of the antheridium. There is a wedge-shaped basal cell which usually does not extend completely across the antheridium (figs. 27, 30). Then there is a succession of oblique walls such as are found in *Osmunda* and *Gleichenia* (figs. 25, 26, 28). These cells, which are fairly regular at first, may become twisted and



FIGS. 25–34.—Figs. 25, 26, young antheridia. Fig. 27, section of young antheridium. Fig. 28, mature antheridium. Fig. 30, antheridium discharging sperms on side away from observer. Fig. 31, empty antheridium with opercular cell. Figs. 32, 33, empty antheridia, side and oblique views. Figs. 29, 34, top view of antheridia.

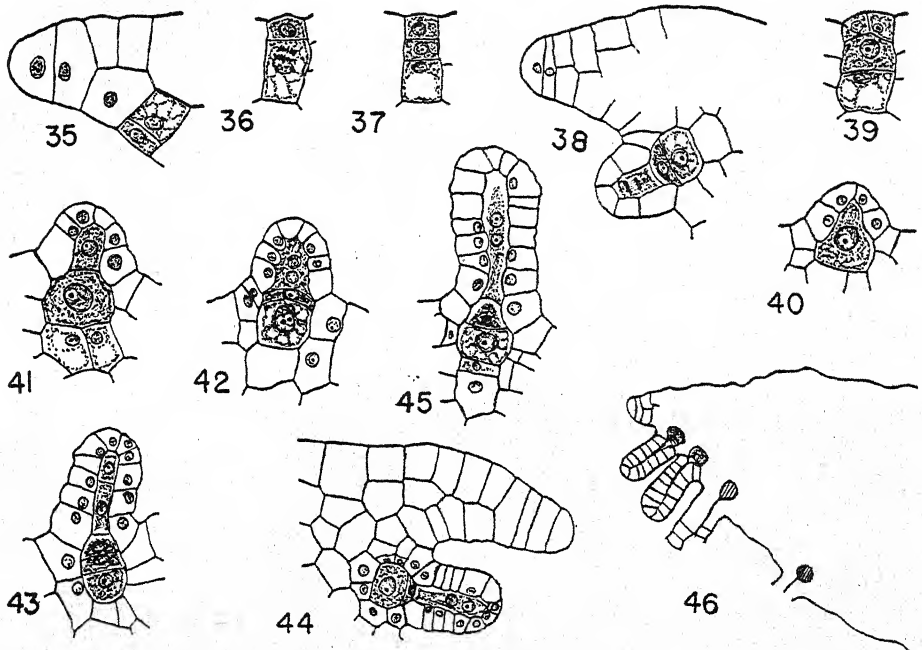
apex (fig. 22). Many slender gametophytes bearing antheridia only are formed under conditions unfavorable for the large monoecious gametophytes, but they are not ameristic. The filamentous, branching, ameristic type of antheridium-bearing gametophyte frequently found in the Polypodiaceae was not found in these cultures. While both antheridia and archegonia are characteristically found on the ventral surface, both may be found on the dorsal. Old gametophytes with the midrib more or less concealed by the ruffled wings are especially likely to have archegonia on the dorsal surface, and irregular gameto-

take peculiar forms with growth of the antheridium (figs. 32, 33). After the formation of the oblique intersecting walls a cap cell is formed which divides once or even twice (figs. 29, 34), forming a small opercular cell which is thrown off with the rupture of the antheridium (fig. 31). On young gametophytes the antheridia may be small but are rarely, if ever, as small as those of the Polypodiaceae. On well-grown gametophytes the number of sperms in an antheridium is large, and the antheridium is usually at least six to eight sperms in diameter, but it may be ten or eleven (fig. 34). The walls have a cutin layer which is particularly heavy

on the basal cell and the lower half of the antheridium. Swimming sperms were found abundantly in these cultures and were just as abundant on regenerated gametophytes as on those which arose from the spore.

The development of the archegonium conforms to that of the type found in leptosporangiate ferns. The initial usual-

gonium might be one of the Polypodiaceae, but in later stages the difference in the neck is evident. The neck is usually much longer than those characteristic of the Polypodiaceae, with usually seven or eight cells in each row, but nine is not unusual. The neck is not only long but the curvature is slight—if any (figs. 44–46). The impression received in examin-



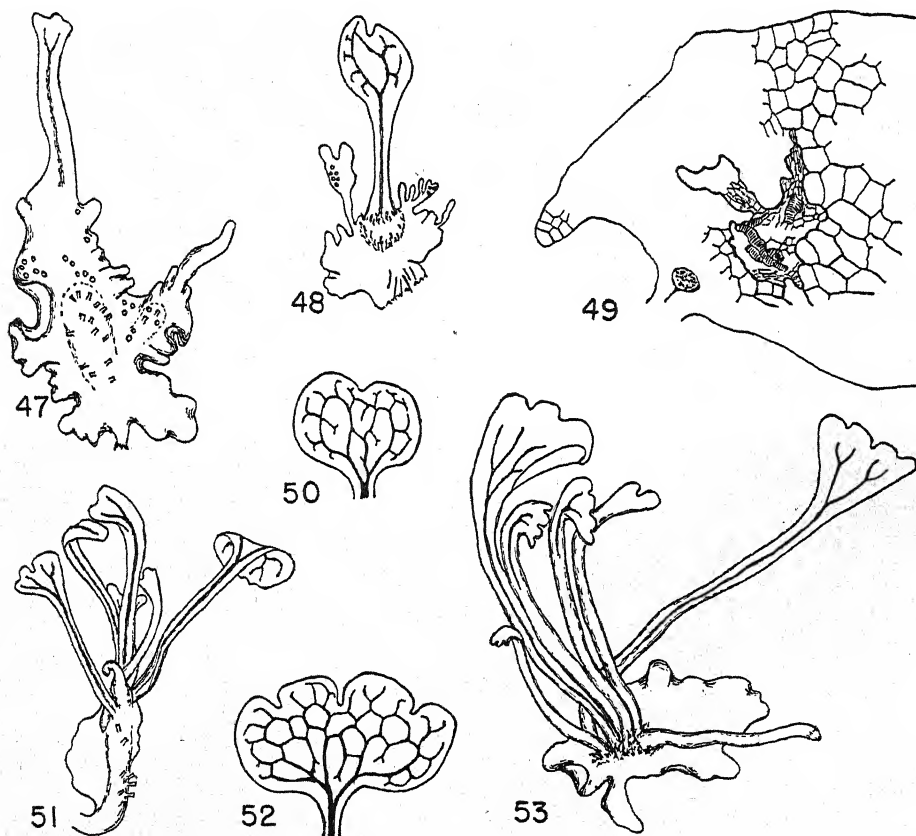
FIGS. 35-46.—Fig. 35, tip of gametophyte with early stage of archegonium. Figs. 36-42, 44, 45, stages in development of archegonium. Fig. 43, abnormal archegonium. Fig. 46, longissection of old thallus.

ly arises close to the apex and the primary neck cell is soon cut off (fig. 35). The characteristic "row of three" is formed, and division occurs in the primary neck cell (figs. 36, 37, 39). There is a vigorous enlargement of the central cell as it pushes between the neck cells and then a wall cuts off the neck canal cell (figs. 40, 41). The axial row is completed long before the neck has attained its full number of cells (figs. 38, 42). At the stage shown in figure 42 the arche-

ing fresh material of old gametophytes is of strikingly long necks, which, on the younger part of the thallus, are pointed forward or in a forward direction but on the older parts are directed downward or laterally and are most abundant on the flanks of the midrib, with relatively few in the center (fig. 23*b*). At the time of maturity of the archegonium, when it is ready to open and fertilization might be expected, the tip of the archegonium points in a forward direction, with its

long axis approximately parallel to the dorsal surface (fig. 44) or making an angle which is usually less than 30° . In most archegonia observed there was no difference in the number of cells on the anterior and posterior sides, although there was sometimes a difference in size

had two neck canal cells and two additional nuclei not separated by a wall. The formation of four neck canal nuclei, which is not unusual in the Cyatheaceae and Dicksoniaceae, did not occur in any of the material sectioned. Although archegonia were formed abundantly on



FIGS. 47-53.—Fig. 47, gametophyte bearing antheridia, archegonia, and apogamous leaf. Fig. 48, gametophyte with antheridial lobe and apogamous leaf. Fig. 49, longisection of gametophyte with xylem elements. Figs. 51, 53, gametophytes with apogamous sporophytes. Figs. 50, 52, young leaves of apogamous sporophytes.

of cells, especially at the base. The surface of the necks is usually more or less cutinized. Cases of anomalous structure of the archegonium were few. On one gametophyte one archegonium had two ventral canal cells and a wall between the neck canal nuclei (fig. 43), while another

both old and young gametophytes, and sperms were also abundant, no cases of fertilization were found and no embryos seen in sections of the gametophytes, nor any young sporophytes of even probable syngamous origin. Ordinarily in cultures where sperms are abundant one is likely

to find at least a few embryos in sections and an occasional syngamous sporophyte in the cultures.

APOGAMY

Apogamy occurred very freely in these cultures. The first apogamous structures appeared when the cultures were a year old, and production is continuing to the present time. In some cases, as in the 1-year-old gametophyte shown in figure 47, the apogamous growth arose as a gradual extension of a relatively thin thallus into a leaf with a well-defined vascular system. In other cases the transition was more abrupt, with the transformation of the apex into a thickened mass which bore glandular sporophytic hairs and continued into an apogamous leaf (fig. 48). As shown in figures 47 and 51, a gametophyte which has been bearing antheridia and archegonia may become apogamous. Marginal lobes bearing antheridia were found on gametophytes with apogamous leaves (fig. 48), and a gametophyte was found which was bearing an apogamous leaf as well as regenerated branches which had both antheridia and archegonia. Under favorable conditions a succession of leaves appeared on the apogamous growth and ultimately a stem and root or roots (figs. 51, 53). The first root was late in developing and usually appeared on the dorsal side of the gametophyte. The sporophyte shown in figure 51 had a slightly larger root on the dorsal side of the thallus. The first leaf showed the forking of the petiole bundle characteristic of *D. conjugata*, and at an early stage there could be seen the anastomoses and network characteristic of the later leaves (figs. 48, 50, 52). Well-rooted sporophytes which were probably apogamous were transferred to soil, but none survived the perils of early growth beyond the leaf height of 7-8 cm.

In only one thallus was there found the type of apogamy shown in figure 49—the development of xylem elements and small-celled sporophytic tissue in the midst of the large-celled gametophytic tissue of the cushion. In general, apogamous structures were more common on thin prothalli than on thick. The apogamous structures were never associated with abortive archegonia. Perhaps in these cultures apogamy might be regarded as obligate, since growth conditions or inherent habit did not favor the formation of syngamous embryos, although there was abundant production of archegonia and antheridia.

Discussion

The preceding account shows that the gametophyte of *D. conjugata* bears a much closer resemblance to the gametophytes of *Gleichenia* and the primitive ferns than to those of the Polypodiaceae. In the experience of the writer, gametophytes of the cordate type show little if any difference within the limits of a genus, particularly in small genera. In the case of a genus as small and as sharply defined as *Dipteris*, it is probable that the gametophyte of *D. conjugata* is representative of the genus.

Germination in *Dipteris* is of a primitive type, consisting as it does of the formation of a plate, occasionally of a mass, and rarely of a filament. The plate and mass type are found only in primitive families, such as the Marattiaceae, Osmundaceae, and Gleicheniaceae. The only published account of germination in *Gleichenia* is that of RAUWENHOFF (9), who described two types—germination resulting in a mass and germination resulting in a filament, with considerable irregularity and variation in both. The writer has had a few species of *Gleichenia* in cultivation from time to time and has

found that plate and mass germination occur in some species, and that there may be also the formation of a broad base like that in the Cyatheaceae and Dicksoniaceae, but that the tendency to form a filament is much weaker than in the Polypodiaceae. The mature gametophytes of *D. conjugata* developed in culture show the same habit of growth as those from Mount Ophir—a notably thick midrib and wings with a high degree of ruffling. This is the type found in at least several species of *Gleichenia*. The antheridia are quite unlike those of the Polypodiaceae. They differ in their greater size, in the larger number of cells found in the wall, and in their large sperm output. The large output of the antheridium is in contrast with that of the *Dipteris* sporangium, which has an output of only sixty-four spores. The antheridia of the Polypodiaceae seem to be uniform in the number of cells in the wall, except for the few species which may have a divided cap cell. The antheridia of *Dipteris* suggest the type found in several families of primitive ferns, such as the Osmundaceae and Gleicheniaceae, and are more like those of the Cyatheaceae and Dicksoniaceae than those of the Polypodiaceae. The archegonium agrees in development with that of the Polypodiaceae, Gleicheniaceae, and other leptosporangiate ferns, but the mature archegonium is peculiar in its long straight neck. The straight neck is found in the Osmundaceae, Hymenophyllaceae, and in some of the Cyatheaceae, but in these families it is not usually so long as in *Dipteris*. It is more like the neck of the archegonium in *Gleichenia*, although in some species of *Gleichenia* the neck may be even longer, with a forward curve, but it may be straight.

Dipteris, then, allies itself with the more primitive ferns rather than with

the Polypodiaceae in the characters of the gametophyte: germination, mature habit, structure and size of antheridium, and an archegonium with a long straight neck. The gametophyte of *Dipteris* has more in common with the Gleicheniaceae, with which it is considered to be allied in sporophytic characters, than with the Polypodiaceae, since it does not have the filamentous type of germination, the relatively thin thallus, the symmetrical antheridium with three wall cells and small sperm output, and the archegonium with the short recurved neck. The contribution of the gametophyte to the question of the classification of *Dipteris* indicates that *Dipteris* does not belong to the Polypodiaceae; it emphasizes the appropriateness of the family Dipteridaceae established by SEWARD and DALE on sporophytic characters.

Summary

1. Germination of the spore in *Dipteris conjugata* results in a plate, in a more or less irregular mass, or (more rarely) in a filament.
2. The mature gametophyte has a thick midrib and ruffled wings; it is similar to that of *Gleichenia* in appearance. No hairs are borne on the gametophyte.
3. The antheridium is of a primitive type with a wedge-shaped basal cell and a wall consisting of four to six additional cells. The cap cell divides once or twice, forming an opercular cell which is thrown off at maturity. The antheridium varies greatly in size, and the number of sperms may be considerable. One diameter of the antheridium may show five to eleven sperms.
4. The archegonium develops in the usual manner of the leptosporangiate ferns. The neck is straight and unusually

long; it is directed forward on the younger parts of the gametophyte, but on the older parts it is directed laterally or downward.

5. Apogamy is of frequent occurrence.

6. The evidence given by the gametophyte of *D. conjugata* suggests a closer relationship to the more primitive ferns, particularly the Gleicheniaceae, than to the Polypodiaceae, and indicates that the family Dipteridaceae established on the basis of sporophytic characters alone

is also justified by the structure and development of the gametophyte.

The writer wishes to express her thanks to the members of the staff of the Botanical Gardens at Buitenzorg and Tjibodas, Java, for their generous assistance in facilitating the collection and culture of the material used in this investigation.

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EXPRESSION OF CERTAIN HEREDITARY FACTORS IN YELLOW BERMUDA ONIONS INDUCED BY UNSEASONABLE PLANTING IN THE GREENHOUSE

S. S. IVANOFF

Introduction

Of the commonly grown crop plants, the onion (*Allium cepa* L.) seems to be unusually sensitive to certain changes in the environment. It is a common experience among growers in southern Texas to have a crop of Yellow Bermuda onions lacking uniformity, particularly in amount of bulbing, size, maturity, doubling and splitting of bulbs, and number of seed stems produced. Bulbing in

onions is definitely determined by the photoperiod during the growing season, and under southern Texas growing conditions only a few varieties are able to produce good commercial bulbs (1). Undoubtedly some of the variations are basically due to lack of genetically pure varieties adapted to local conditions. HAWTHORN (2) has shown that inbreeding, combined with selection away from undesirable characters of doubling and

premature seeding, may result in lines of onions free from these characteristics. Recently the same writer (3) has developed through selection from the heterozygous Early Grano onion a new strain, the Texas Grano, which shows a remarkable degree of uniformity as to size, bulbing, and early maturity. Such variations, however, may also be attributed to the influence of local environmental conditions under which the crop is grown and which may remain expressionless under a different environment. For example, the number of doubles and splits—and particularly bolters¹ may vary significantly from season to season, farm to farm, place to place on the same field, and even within the same section of row. Such variations, which depend principally on environment, have been observed in both open-pollinated varieties and inbred lines of Bermuda onions.

This report deals with outstanding variations as manifested by different plant forms and behavior within a population of onions which were induced by unseasonable planting of seed in a greenhouse, where the plants sometimes grew at unusually high temperatures and light intensities. The results were obtained in an attempt to shorten the life cycle of the plant from 2 years to 1 year by various means, as a practical aid in hastening the work on breeding for disease resistance. (Under ordinary conditions, the bulb crop in southern Texas is harvested in April or May. To obtain seed from the bulbs; the latter need to pass through a rest period in storage during the summer, after which they are planted in November. Seed from these bulbs is obtained in May or June of the following year. Under these conditions of culture, the onion plant completes its life cycle in about 22 months.)

¹ Plants which form seed stalks prematurely.

Differences in bulbing within a population in response to definite sets of controlled environmental conditions in the onion have been recorded by other workers. McCLELLAND (5) studied different onion varieties in their response to different photoperiods and found that, while one variety may bulb 100% under a certain photoperiod, in another variety only certain individual plants may bulb under the same photoperiod. MAGRUDER and ALLARD (4) and WILSON (9) recorded similar results. MAGRUDER and ALLARD worked with eighteen varieties grown under various photoperiods and found that Yellow Bermuda was the only variety that produced normal bulbs under the 10-hour light treatment, but only 10% of the plants within the population were able to do so. With longer photoperiods other varieties performed similarly, as some plants within the group formed good bulbs while the rest of the plants failed to bulb. WILSON, studying the influence of hydrogen-ion concentration on growth of onions, found that within a given reaction series bulbing occurred first on the least thrifty plants. The work of ROBERTS and STRUCKMEYER (6), because of its emphasis on variations within the same population of plants, has closer relation to the studies here reported. They investigated the influence of temperature and light periods on some forty-three species and varieties and found that seedlings, and in some cases both seedlings and clones, may have either uniform or variable populations—depending upon the environment in which they were grown. For example, plants of pennycress (*Thlaspi arvense*) grown from seed appeared more uniform in size and other characteristics in a cool, long-day environment than in a cool, short-day environment. On the other hand, clones

of *Rudbeckia laciniata* were more variable in cool than in warm photoperiods. However, since they found that clonal populations were also subject to similar variations as the seedling populations under certain conditions, it is not likely that these variations were due in all cases to genetic diversities. It must be concluded that other factors influenced variability. The same workers (7) later reported that Chinese cabbage showed a relatively uniform type of growth in cool temperatures, particularly on long photoperiods, but showed quite variable growth in warm temperatures, particularly on short photoperiods. They collected seed (open-pollinated) from the different types of plants from the same population. The seedlings from these types, however, did not show the differences of the parents when grown in a cool location, but they did tend to be like the parent plants when grown in a warm location. Recently YARNELL (10) has summarized the work pertaining to the influence of environment on the expression of hereditary factors as related to problems of breeding in southern United States.

Procedure

Two plantings of onions grown from the same lot of seed were compared. One lot was grown during the usual period for onion production in the Winter Garden region and the other was raised during the summer in the greenhouse. For the seasonable crop, seed was planted in an open seedbed in early fall, the seedlings transplanted in late November or December, and the mature bulbs harvested the following April. With the unseasonable plants the seed was planted in June in a greenhouse where the temperatures often reached 125° F. or higher and were 4°-35° higher than outside. The greatest differences in temperature between the

two crops occurred during the summer months when the greenhouse plants were in their seedling stage, and again in the following spring when the bulbs were maturing. In the case of the seasonably planted onions the highest average temperature occurred as the bulbs reached maturity.

There were two experiments of this kind, each 1 year in duration. The second experiment was conducted 1 year after the first and was intended to be essentially a repetition of the first. In the first experiment some of the unseasonable plants were transplanted into jars of soil or to the field to avoid crowding. A third experiment, conducted from October, 1943, to April, 1944, was designed to test the behavior of the second-generation plant progenies derived from some of the plant material grown in the first trial.

Seed of the Yellow Bermuda variety was used: one lot of a first generation inbred line and a second lot of an open-pollinated commercial stock. The photoperiods during the summer and winter solstices at Winter Haven, Texas, where the trials were made, are approximately 14.1 and 10.2 hours, respectively.

Experimental results

UNSEASONABLE PLANTS

Seed of Yellow Bermuda (first-generation inbred) onions was planted on June 1, 1942, in a greenhouse bench in uniform, light sandy loam. Abundant space was provided to avoid crowding of the young plants. There were forty plants in all. Following emergence, the plants grew normally for several weeks, after which some of them began to bulb and finally formed sets,² at which time the

² Onion sets are merely undersized (usually not more than $\frac{3}{4}$ inch in diameter) mature onion bulbs raised from seed and arrested in growth. Sets re-

leaves withered. The sets matured at different times (about 55-120 days after planting the seed) and varied from 8 to 37 mm. in diameter. There were twenty-two sets formed, or 55% of the total number of plants. The sets were harvested as they matured, and after passing through a rest period for 6-8 weeks they were planted in the fall and the winter; some were planted in the field and others in jars of soil kept in the greenhouse. All these sets developed into single-bulb plants in April and May of the following year, without seed stalks or seed.

The other plants which did not form sets (referred to hereafter as non-set-formers) appeared as normal young green plants with erect turgid tops and no conspicuous swelling at the base. After some time, however, certain significant variations in form were distinguished among them. Thus, on December 1, 183 days after planting, in addition to the sets already mentioned, there were two other distinct forms: plants which had not yet started to bulb and plants which had formed large bulbs, reaching their ultimate size of $3\frac{1}{2}$ -4 inches or more in diameter (fig. 1A-C). Several months later, at harvest in the following spring and summer when all plants had reached maturity, there were altogether three distinct plant types: (1) 25% were large single bulbs which matured without producing seed stalks; (2) 50% were large multiple bulbs (two to five per plant) that had matured without producing seed stalks; and (3) 25% were multiple bulbs with seed stalks bearing large normal-sized umbels and

sume growth when planted following a short rest period. Ordinarily they are produced in the northern states by sowing the seed thickly so that the bulbs reach their maximum growth quickly, usually within 3-4 months after planting the seed (8). Sets have been produced in southern Texas in similar manner.

viable seeds. All the plants matured in less than a year after planting the seed, and some of them (type 3) completed their entire life cycle from seed to seed within that time. A diagrammatic presentation of the various onion forms produced at different times is shown in figure 2.

SEASONABLE PLANTS

Following the usual practice of raising onions in this region, seed was planted in an outdoor seedbed on August 28, 1942, the plants were moved to the field on November 24, and the bulbs harvested in late April of the following year. These were considered as control plants. Two kinds were used: 201 plants raised from the same inbred lot of seed as was used in the greenhouse planting, and 477 plants raised from open-pollinated commercial Yellow Bermuda seed. The latter were included in this trial for the purpose of comparing the behavior of inbred-line onions with that of an open-pollinated variety. At the end of the season, 98% of the inbred-line onions were single bulbs, 1% were bolters, and 1% were doubles; of the open-pollinated onions, 94% were single bulbs, 1% were bolters, and 5% were doubles. The difference in behavior of the two kinds of control plants was relatively slight, but the difference between both kinds and the greenhouse plants was striking. The differences were evident mainly in the type of plant forms produced. The field-planted seed did not produce sets, and none of the plants developed into multiple-bulb, seed-producing types—as was the case with the greenhouse plants. On the other hand, 1% of the control plants were bolters, and none of this type occurred among the greenhouse plants.

There are significant differences in appearance between the bolters found in

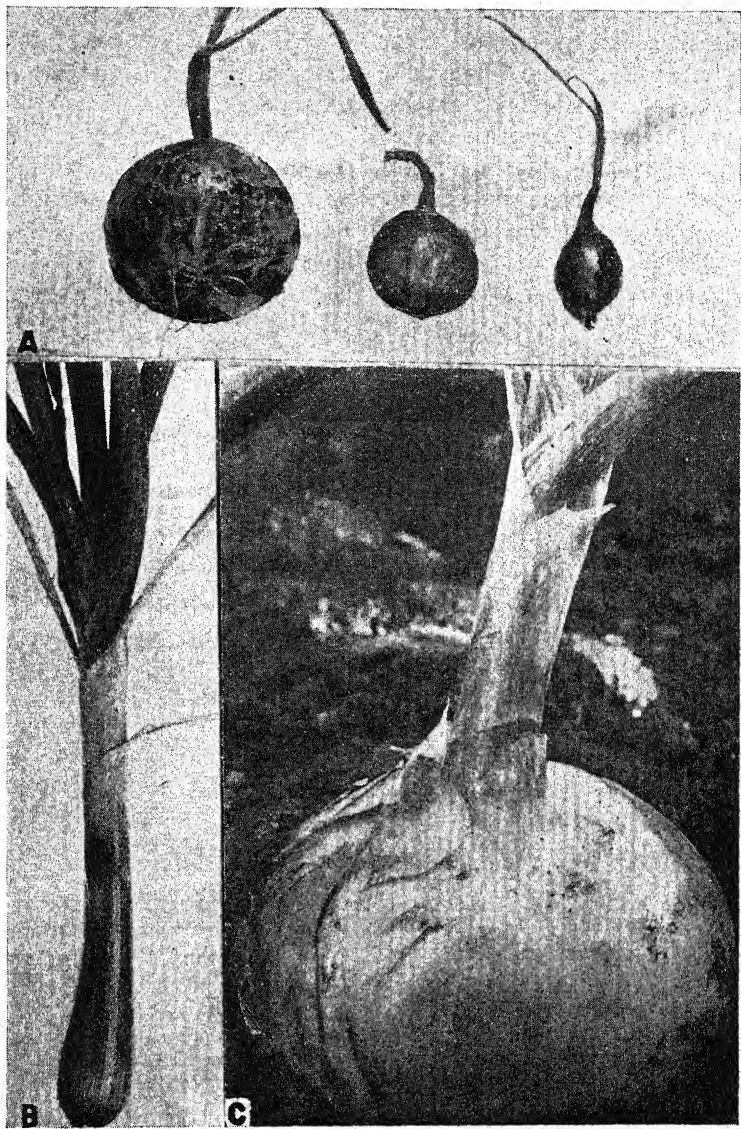


FIG. 1.—Three variations in Yellow Bermuda onions as they appeared on December 1, 1942, 183 days after planting the seed unseasonably in greenhouse: A, sets of various sizes; B, bulbless plant; C, large bulb form nearing its ultimate size. Several months later, variations shown at A developed into large single bulbs; variations at B developed into three types (single bulbs, multiple bulbs without stems, and multiple bulbs with several seed stems bearing viable seeds); and variations at C developed into multiple bulbs without stems or multiple bulbs with seed stems bearing viable seeds. Twenty-five per cent of the non-set-formers (B and C) completed their life cycle in less than a year. $\frac{3}{4}$ natural size.

the field and the multiple-bulb, seed-bearing plants of the greenhouse lots. As a rule, the bolters were single-bulb plants with one seed stalk which usually arose through the center but occasionally occurred on the side of the bulb. The bolters had relatively small umbels and under Winter Haven conditions rarely produced viable seed. On the other hand, the appearance and manner of develop-

shoots, each of which later forms a bulb. Each of these bulbs may in time produce one or more stalks with large umbels and abundant seed.

Another experiment, essentially like the first, was conducted the following year. However, open-pollinated Yellow Bermuda seed was used both in the greenhouse and in the field. The soil was of a heavier type than that in the first experi-

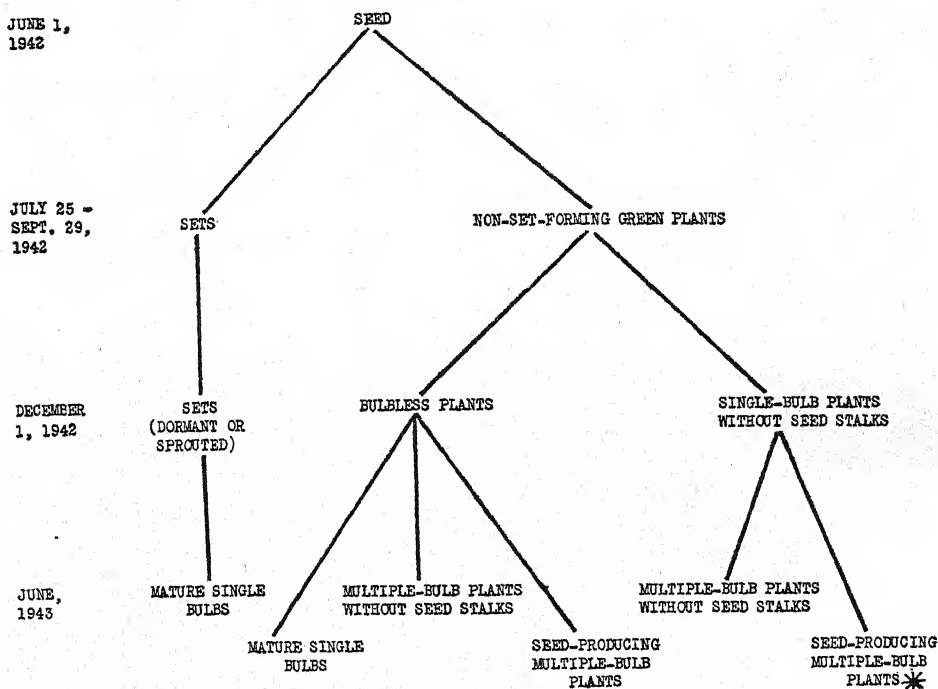


FIG. 2.—Diagrammatic presentation of various onion forms produced at different periods from seed planted unseasonably in greenhouse (experiment 1). (*Seed from one plant of this type, when planted seasonably in the field, produced 92% single bulbs, 8% doubles, and no bolters.)

ment of the unseasonable multiple-bulb, seed-producing plants which completed their life cycle in less than 1 year were indistinguishable from those of the commercially grown seed-producing plants at the end of their second-year period. With these 2-year-cycle plants, which are raised from large bulbs grown the season before, the "mother-bulb" before disintegrating produces several or more

Seed was planted in the greenhouse on June 20, 1943. Ample space was again provided for the emerging plants in order to avoid conditions favorable for set formation. All seeds were planted at a uniform depth of $\frac{1}{2}$ inch. Of the thirty-two plants obtained, 53% formed small sets by the middle of September, after which no more sets were formed. The rest of the plants developed as stout

seedlings and continued to grow without bulbing for some time. These plants were left in place in the greenhouse bench until the experiment was completed, and they finally developed into multiple-bulb forms, all but two of which (87%) produced viable seed in May, 1944. Like the plants of the first experiment, they also completed their life cycle in less than a year. After a rest period the sets were

gle-bulb bolters, and 3% doubles. There were no multiple bulbs produced in the outdoor planting. The results of the two experiments are summarized in table 1.

A third experiment was conducted to determine what kind of plants would develop from the seed produced by the plants grown in the greenhouse which completed their life cycle in 1 year (type 3), when the seed is planted in season in

TABLE 1

TYPES OF YELLOW BERMUDA ONION PLANTS PRODUCED BY UNSEASONABLE PLANTING AND SUMMER CONDITIONS IN THE GREENHOUSE, CONTRASTED WITH FIELD PLANTING IN SEASON, AT WINTER HAVEN, TEXAS

STRAIN OR VARIETY	LOCATION AND DATE OF SEED PLANTING	TOTAL NO. OF PLANTS	SETS PRODUCED (%)	TYPE DEVELOPED FROM SETS (%)			TYPE DEVELOPED FROM NON-SET-FORMERS (%)		
				Single bulb	Multiple bulb without stems	Multiple bulb seed-producers	Single bulb	Multiple bulb without stems	Multiple bulb seed-producers
Inbred.....	Greenhouse June 1, 1942	40	55	100	0	0	25	50	25
Inbred.....	Field Aug. 28, 1942	201	0	99*	1†	0
Open-pollinated commercial.....	Field Aug. 28, 1942	477	0	95*	5†	0
Open-pollinated commercial.....	Greenhouse June 20, 1943	32	53	100	0	0	0	13	87
Open-pollinated commercial.....	Field Oct. 5, 1943	532	0	97†	3‡	0

* 1% single-bulb bolters.

† Including 0.3% single-bulb bolters.

‡ All were doubles (two-bulb plants) and splits.

planted again, some in the greenhouse and others in the field. All of them developed to maturity as single bulbs, as was the case with the sets in the first experiment.

For controls in this experiment, there were 532 plants raised from seed planted in an outdoor seedbed on October 5, 1943, and transplanted to the field on December 24. In the following April, when the bulbs were harvested, there were about 97% single bulbs, 0.3% sin-

gle-bulb bolters, and 3% doubles. There were no multiple bulbs produced in the outdoor planting. The results of the two experiments are summarized in table 1.

Seed from one of these plants of the first experiment was planted in the seedbed October 5, 1943, a few months after it was harvested. The plants were transplanted to the field on December 22 and harvested when the bulbs matured (during the latter part of April, 1944). There were 110 plants in all. For checks there were 206 plants of open-pollinated com-

mercial Yellow Bermuda which were raised at the same time and handled in the same manner as the first lot. The check plants were grown in two rows, one on each side of the row where the above-mentioned lot of plants were grown. The results at harvest showed no bolters among the progenies of the type-3 plant and only 0.4% of bolters in the check plants. The low percentage of bolting in the commercial open-pollinated Bermuda is an indication that the environmental conditions were not very favorable for bolting. On the other hand, there were 8% of doubles in the type-3 progenies and 3% in the checks. These results show no significant difference in regard to amount of bolting between the two lots of plants. Also, the progenies of the type-3 plants were not particularly inclined to bolt under the conditions of the experiment.

Discussion

The results reported pertain to the influence of an unusual environment on the hereditary behavior of the Yellow Bermuda onion. The high temperatures, long photoperiods, and high light intensities in the greenhouse during the summer are associated with the development of various plant forms which are seldom if ever encountered in seasonably grown onions in the field. Some seedling plants interrupted their development by forming sets; others continued their growth without interruption and developed at different times into various forms—such as single bulbs, multiple bulbs without seed stalks, and multiple bulbs which produced seed. Some of these forms were entirely absent in comparable lots grown under lower temperatures, short photoperiods, and lower light intensities of the normal onion-growing season.

In interpreting these results, and in

similar studies by other workers, the degree of genetic diversity of the plants employed should perhaps be considered. The inbred line of onion seed may be assumed to be genetically less heterozygous than the open-pollinated lot, but, in view of the results obtained, its uniformity apparently was not of a high degree. On the other hand, the open-pollinated seed stock may be expected to be genetically more variable and is likely to give rise to a number of different genotypic lines, as the onion plant is partly self-pollinated and partly cross-pollinated. As a consequence, within a lot of commercial open-pollinated seed there may be individual seeds which are the result of hybridization between two plants and others which are of inbred nature. Some of the lines arising from such seed will be first-generation inbreds, while others, progressively fewer in number, may be second-, third-, or even later-generation inbreds. Since onions lose vigor by inbreeding, and since vigor—according to observations made by this writer—seems to be associated with a tendency for doubling and bolting under field conditions, it may be possible that the factors for hybrid vigor are partly responsible for the development of some of the variations in these trials. Perhaps in all similar studies the factor of the degree of cross-pollination of the experimental plant species should be taken into consideration.

Some applications of these results to onion breeding seem evident. The shortening of the life cycle of the onion from 2 to 1 year would be of great help to the breeder, provided the plants which complete their life cycle in 1 year are not segregates for the bolting habit in the field. It is possible that under certain conditions of culture perhaps all the in-

dividuals of a certain population may shorten their cycle to 1 year, as the results of the second experiment indicate. In this experiment 87% of all the non-set-formers completed their life cycle in less than a year, as compared with only 25% of the same group of plants in the first trial. These differences in results between the two trials may be accounted for in part by the differences in environment of the two seasons.

Even commercial application of these results may be suggested, in that seed may be produced in one growing season in certain areas by planting early and directly in the field. Such practice would considerably reduce the cost of seed production. Recently a seed-to-seed crop of Yellow Bermuda in 1 year was produced unintentionally by growers near El Paso, Texas, by direct planting of seed in September. Under the favorable conditions provided approximately 90% of the plants went to seed, but the great majority of the "seeders" had only one seed stalk and the yield of seed was light. By further experimentation, such as planting the seed more thinly or thinning out the plants early in the season, it may be possible to induce all or nearly all the plants to seed in 1 year and to produce several seed stalks. Recently, such attempts have been made by commercial seedsmen elsewhere. However, a word of caution in regard to such practice is necessary. Since not all plants may go to seed, it is likely that in the course of several generations the genetic composition of the onion variety may be altered to such an extent—particularly in regard to bolting—as to make it undesirable for raising bulb crops. It would be necessary in such cases to raise and maintain a foundation seed stock of the original variety. Certain varieties other than

Bermuda, such as Early Grano, however, are known to bolt either not at all or only negligibly under seasonable conditions in some regions. With these varieties the chances of changing to a bolting type would be correspondingly less than in case of the Bermuda onion.

The studies on the influence of environment on the expression of hereditary factors, as far as the practical breeder is concerned, point to the conclusion that the breeding of superior varieties of plants should be done in the region where the variety is intended to be grown commercially. The aim of the plant breeder should be to bring out and fix those genetic characters of a plant which would best enhance its economic value as a commercial crop in a particular locality.

Summary

1. Studies are reported from two trials on outstanding variations in form and behavior among individuals within a population of Yellow Bermuda onion plants, brought about by unseasonable planting (in June) of the seed in the greenhouse, where the plants sometimes grew at unusually high temperatures and light intensities. These developments were contrasted with the lack of similar variations in seasonably grown plants (from fall through spring) in the field.

2. The unseasonably grown plants produced within a year (*a*) onion sets, all of which, planted after a rest period, developed into single large bulbs; (*b*) single bulbs of various sizes; (*c*) multiple bulbs (two to five per plant) maturing without producing seed stalks; and (*d*) multiple bulbs producing seed stalks and viable seed. Seed from the last group of plants, when planted in season, produced mostly single bulbs with no seed stems.

3. From 10 to 40% of all unseasonably

grown plants completed their life cycle in less than a year, forming multiple bulbs and stems with viable seed.

4. The seasonably grown plants in the field produced mostly single bulbs and a few doubles, splits, and bolters.

5. Some practical applications of the results of these studies are suggested.

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EFFECT OF INDOLEACETIC ACID IN INHIBITING STEM ABSCISSION IN *MIRABILIS JALAPA*¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 566

J. M. BEAL AND A. GERALDINE WHITING

Introduction

Several investigators have reported on the effectiveness of certain of the growth-regulating substances in delaying abscission of plant organs (2, 3, 5, 8, 9), an effect of considerable economic importance to many plant growers. A study of the anatomical effect of some of these substances on abscission of the leaves of *Coleus* has been reported by MYERS (8). The abscission of a stem or fruit, however, may differ structurally from the

process which occurs in the leaf petiole, and it is possible that these differences may affect the response to the growth substance. The influence of indoleacetic acid on stem abscission in *Mirabilis jalapa* has been briefly mentioned by HAMNER (4). Further observations on the effect of indoleacetic acid in inhibiting stem abscission in that plant are reported herewith.

METHODS.—The plants used in this investigation were grown during the summer of 1939. Seeds of four o'clock, *Mirabilis jalapa* Linn., were planted in good garden soil in pots and the resulting seed-

¹ This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

lings kept on an evenly lighted greenhouse bench, where they received the care necessary to insure good growth.

Three groups, selected for uniformity of size, were chosen from these plants for the studies. The plants of one lot (group 1) were permitted to grow without additional treatment and constitute the controls. Those of group 2 were decapitated by cutting squarely across the first internode when the second internode had just started to elongate. The length of first internode remaining after decapitation averaged about 0.5 inch. Plants of group 3 were decapitated in the same manner, but immediately after decapitation the cut surface of the internodes was covered with a 2% indoleacetic acid-lanolin mixture. The three groups were subsequently kept under as nearly identical growing conditions as possible.

Material for histological study, consisting of the cotyledonary node with adjacent portions of the hypocotyl below and of the first internode above, was collected at various intervals, fixed in Nava-shin's solution, and carried into paraffin through the tertiary butyl-alcohol method. Sections were cut at 10μ and stained in a modified triple stain.

Observations

GROSS RESPONSES.—The untreated plants of group 1 showed continued development of the main axis from the terminal buds. The axillary buds did not expand noticeably, and there was no indication of abscission of stems in any of these plants during the time of the experiments.

The plants of group 2, which were decapitated and not further treated, responded by marked extension and development of the axillary buds at the cotyledonary node. Generally there were present also accessory buds which de-

veloped at almost the same rate as the axillary ones. Although the decapitated first internodes made no further appreciable growth, they frequently remained green for as long as 2 weeks after decapitation, and then they usually became yellowed and dropped from the plants while still relatively turgid or after becoming much shriveled. Practically all of them had fallen at 3 weeks.

The plants of group 3 which were decapitated and treated with the indoleacetic acid-lanolin mixture behaved in essentially the same manner as has been described by HAMNER. While the growth of buds in the axils of the cotyledons is greatly delayed, they nevertheless grow rapidly after about the sixth day following application of the lanolin mixture. These internodes develop apical tumors and, according to HAMNER, may remain firmly attached for several months.

HISTOLOGICAL RESPONSES.—Longisections of the cotyledonary node of untreated controls showed that growth in diameter continued in these stems (figs. 1A, 2A, B). The small tiered cells at the base of the internodes suggest that elongation is continued in this region. The axillary buds remained unexpanded and essentially unchanged. Abundant starch was stored, chiefly in the pith (fig. 2A, B). No indications of the development of an abscission layer were observed in any of these sections.

The plants which were decapitated and allowed to develop with no additional treatment behaved in the same manner as has been noted by both LLOYD (6) and HAMNER. There is neither increase in diameter of the internodes following decapitation nor additional secondary thickening. As early as 3 days after decapitation these internodes showed shrunken and collapsed cells in the pith, resulting in the formation of cavities (fig.

1B). Active growth had already begun in the axillary buds at this time, but cells of the hypocotyl and of the cotyledonary node seemed unaffected.

upper one extends entirely across the base of the internode while the lower extends outward only as far as the outer ring of vascular bundles. In sections cut

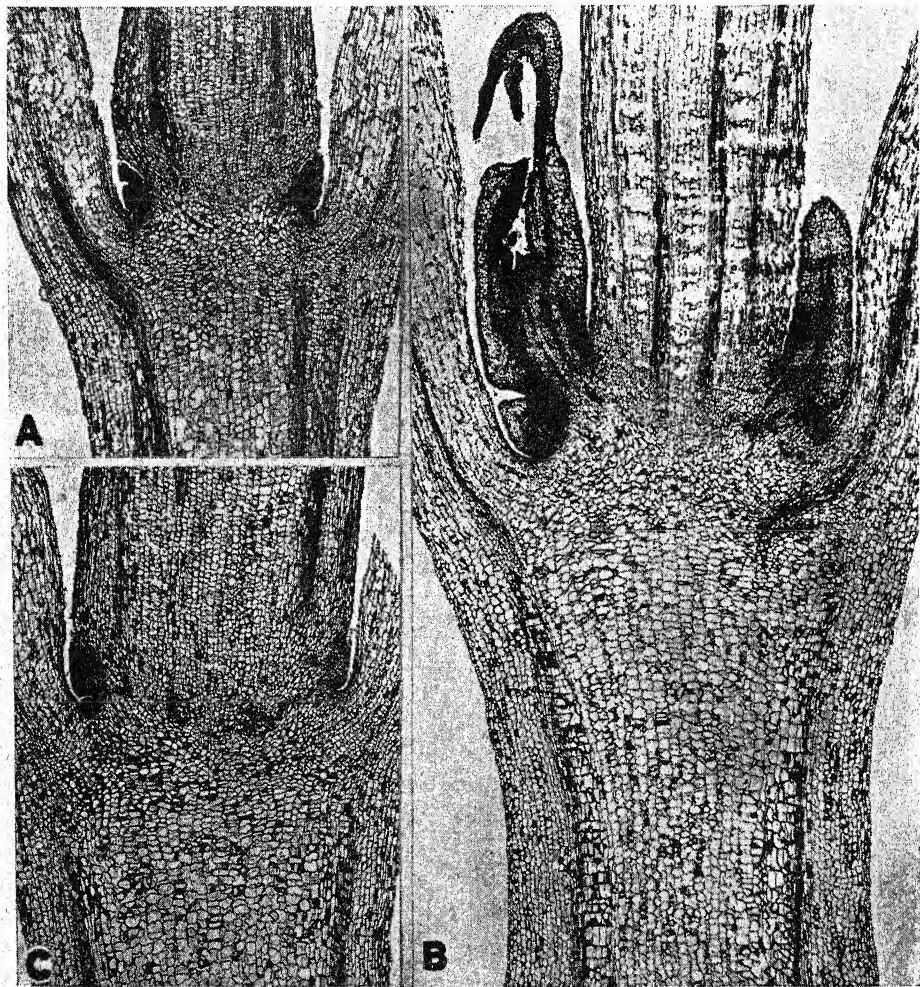


FIG. 1.—Longisections through cotyledonary node: *A*, control plant showing axillary buds and general turgid condition throughout; 8 days after beginning experiment. *B*, decapitated plant without indoleacetic acid, showing cavities in internode resulting from shrinkage and collapse of cells, and developing axillary shoots; 3 days after decapitation. *C*, plant decapitated and treated with indoleacetic acid, showing lateral buds inactive, internode larger in diameter, and cells generally turgid; 3 days after decapitation and treatment.

The earliest indication of an abscission layer was found at 14 days following decapitation (fig. 3*A, B*). Here two meristematic regions are clearly apparent; the

in the cotyledonary plane this zone connects with the vascular bundles of the axillary shoots, while those cut in the intercotyledonary plane show connection

with the outer bundles ascending from the hypocotyl. While the lower meristem has been observed in a number of stems in which no indication of the upper could be detected, there is no evidence pointing to its direct participation in abscission. Whenever the upper of these two regions has been found, the lower has been present without exception. It clearly is not a part of the actual abscission layer, however, but just what its relation may be to abscission or other associated activities is not known.

Meanwhile, the axillary buds, and usually also the accessory buds, have been developing rapidly (fig. 4A, B). The diameter of the decapitated internodes remained relatively unchanged. Many of the internodes had shed by the eighteenth day following decapitation, but others remained for more than 24 days—at which time the experiments were terminated. In none of these plants was it possible to demonstrate storage starch, as was possible in the controls.

An interesting, and perhaps the characteristic, condition immediately preceding abscission is shown in figure 7. The internode, although apparently not materially shrunken, had not increased in diameter. Collapse and disintegration of cells, especially in the pith, resulted in the formation of conspicuous cavities. Continued growth in diameter of the hypocotyl and cotyledonary node had set up considerable differential tension between the latter region and the base of the internode. When an abscission zone developed, the break appeared to begin at the surface region of the internode as a result of the differential tension. Many of the cells at the base of the internode became crushed and appeared as a black line extending across the stem (fig. 7A, B). On the slides these cells stain intensely with orange G. When the internode

abscised, these crushed cells appeared to remain attached and were carried away with it, since none remained on the stems examined (fig. 6). Subtending the crushed cells is a zone of suberized cells, and it is at the upper margin of this zone that the actual break appears to occur in the process of abscission in *Mirabilis*. The line along which the break occurs has been called the "separation layer" by LLOYD, who reported it to consist of one to five tiers of cells in older organs, such as internodes. Our material shows that it comprises at least five tiers of cells, possibly more (figs. 6, 7), for the line of separation is highly irregular.

The lower or proximal portion of the abscission zone consists of meristematic cells which proliferate freely during and following abscission (figs. 6, 7). This region functions as a phellogen, although it comprises a number of layers or tiers of cells.

Lying across the stelar portion of the stem, between the abscission zone and the lower meristem, is a zone of parenchymatous cells which apparently remain relatively unchanged during all this time. While divisions, associated with the growth in the cotyledonary zone, occur in some of these cells, the group as a whole appears to play no active role in the process of abscission.

Plants which were decapitated and treated with the indoleacetic acid-lanolin mixture (fig. 1C) showed certain similarities to the controls. As noted earlier, the internodes increased in diameter and showed essentially no shrinkage or collapse of cells in the pith or other regions. And while the buds in the axils of the cotyledons were inhibited or definitely delayed in development, they generally grew rapidly, beginning about the sixth day following treatment (fig. 5A). None of these plants showed evidences of an

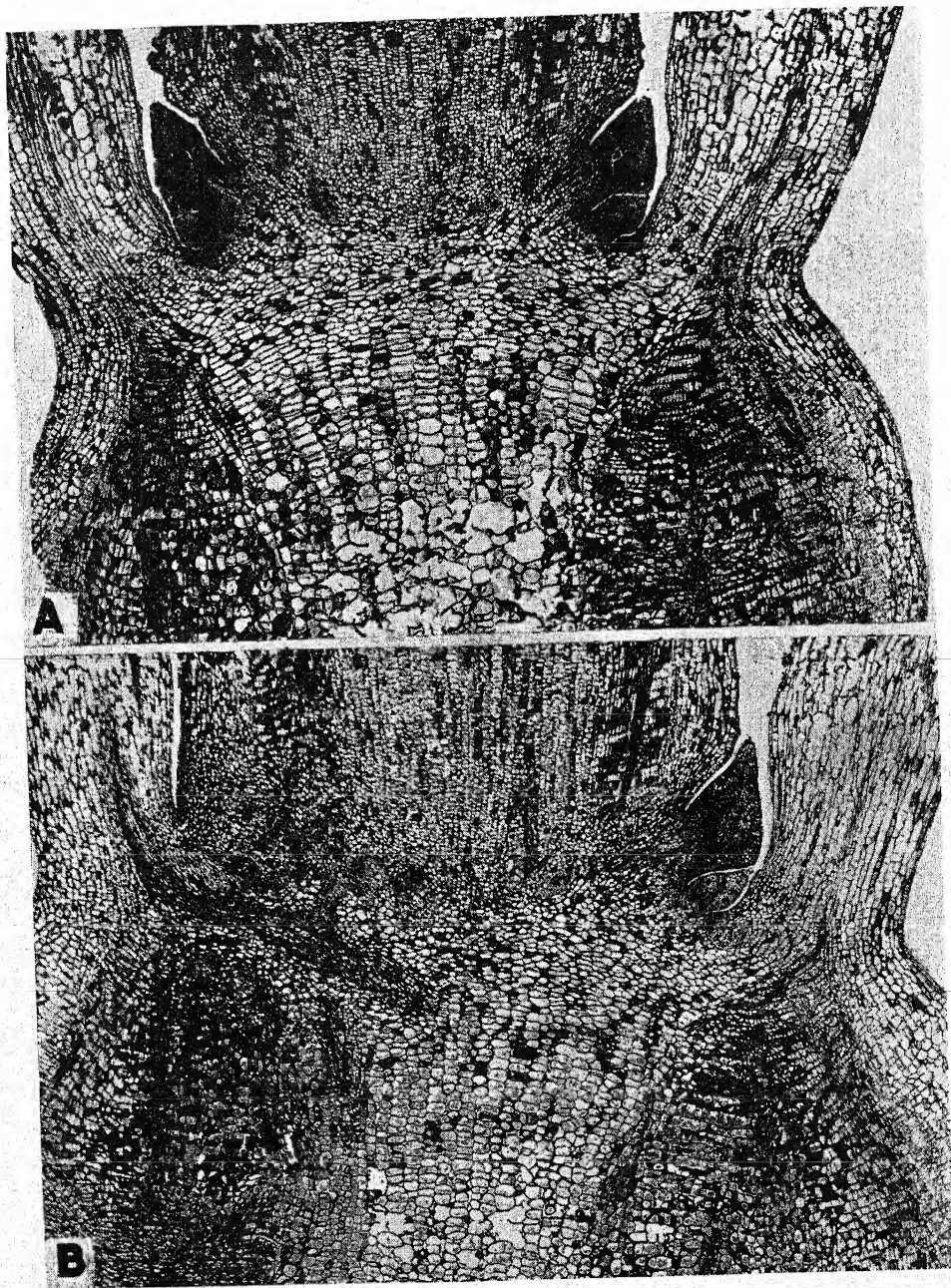


FIG. 2.—*A*, longisection of control; 18 days after start of experiment. *B*, same at 24 days; axillary buds still inactive, cells throughout sections generally turgid, those of pith and rays containing starch.

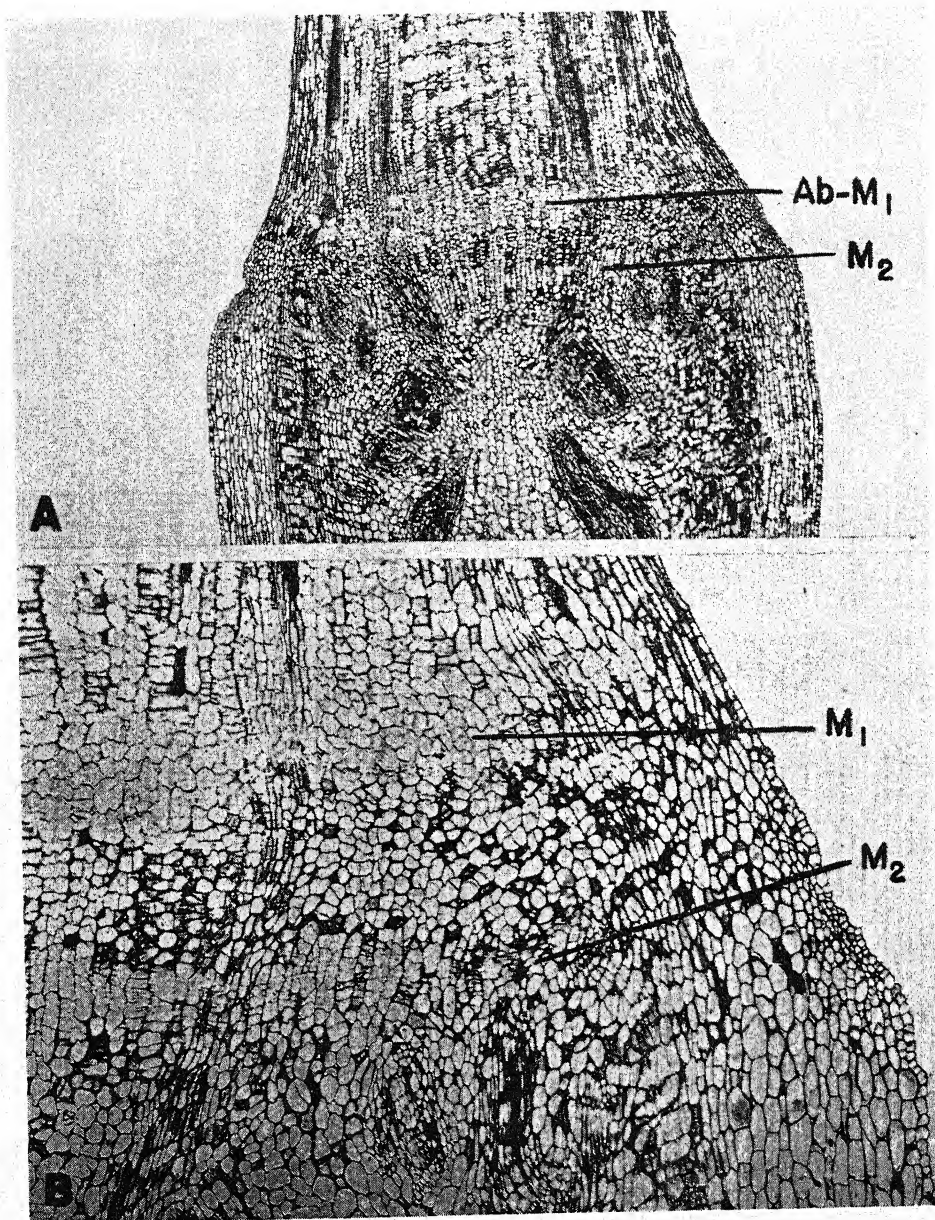


FIG. 3.—*A*, longisection of decapitated plant through intercotyledonary plane, showing developing upper meristematic region or abscission zone ($Ab-M_1$) and lower meristematic zone (M_2); *B*, same at higher magnification to show details. Fourteen days after decapitation.



FIG. 4.—A, longsection of decapitated plant with well-developed abscission layer and lower meristematic zone; pith shows shrunken cells and cavities; axillary shoots have greater diameter than internode; B, same section showing details of abscission and lower meristematic zones. Eighteen days after decapitation.

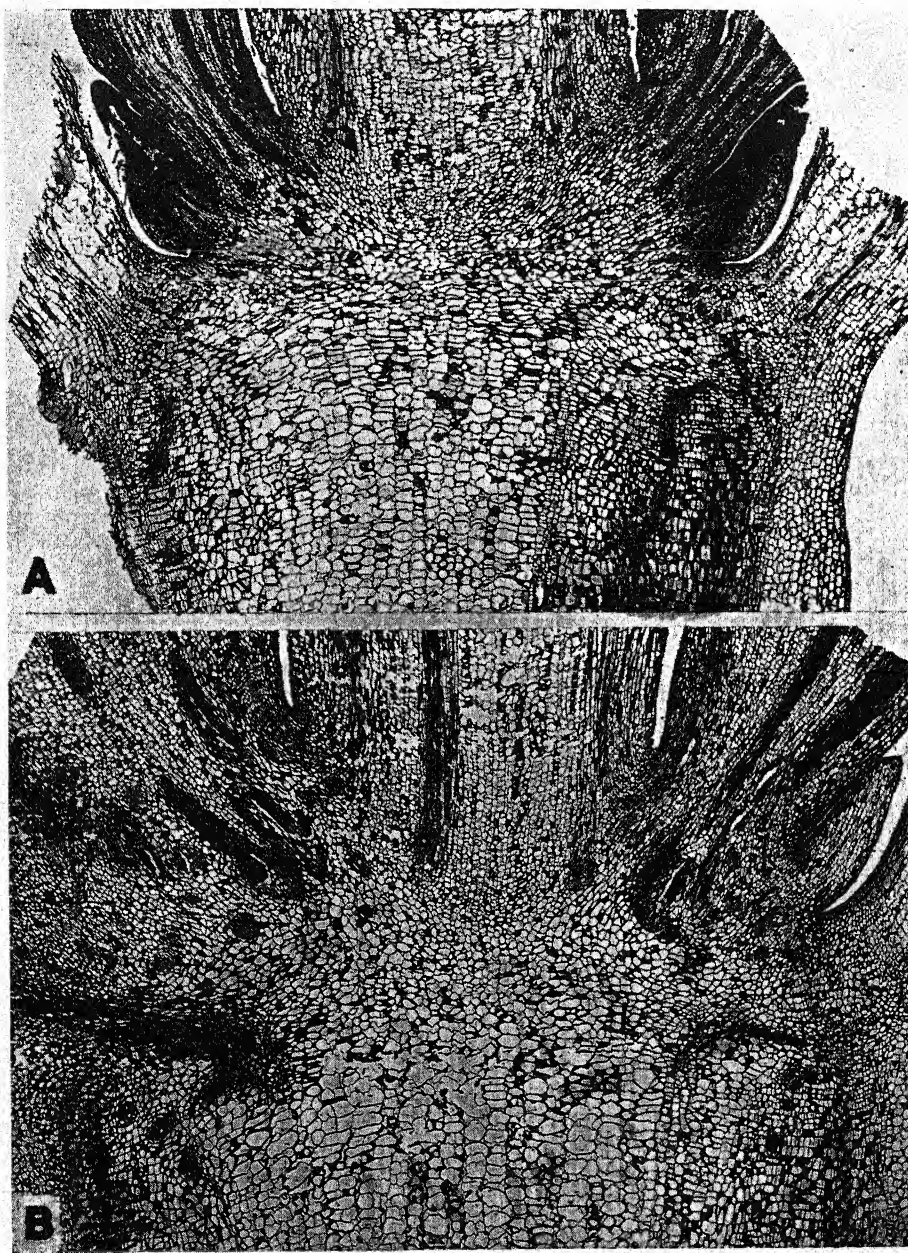


FIG. 5.—*A*, longisection of plant decapitated and treated with indoleacetic acid at 14 days. *B*, from plant treated in same way at 24 days following treatment; cells generally turgid, axillary shoots prominent, and internodes increased in diameter; no indication of abscission zone.

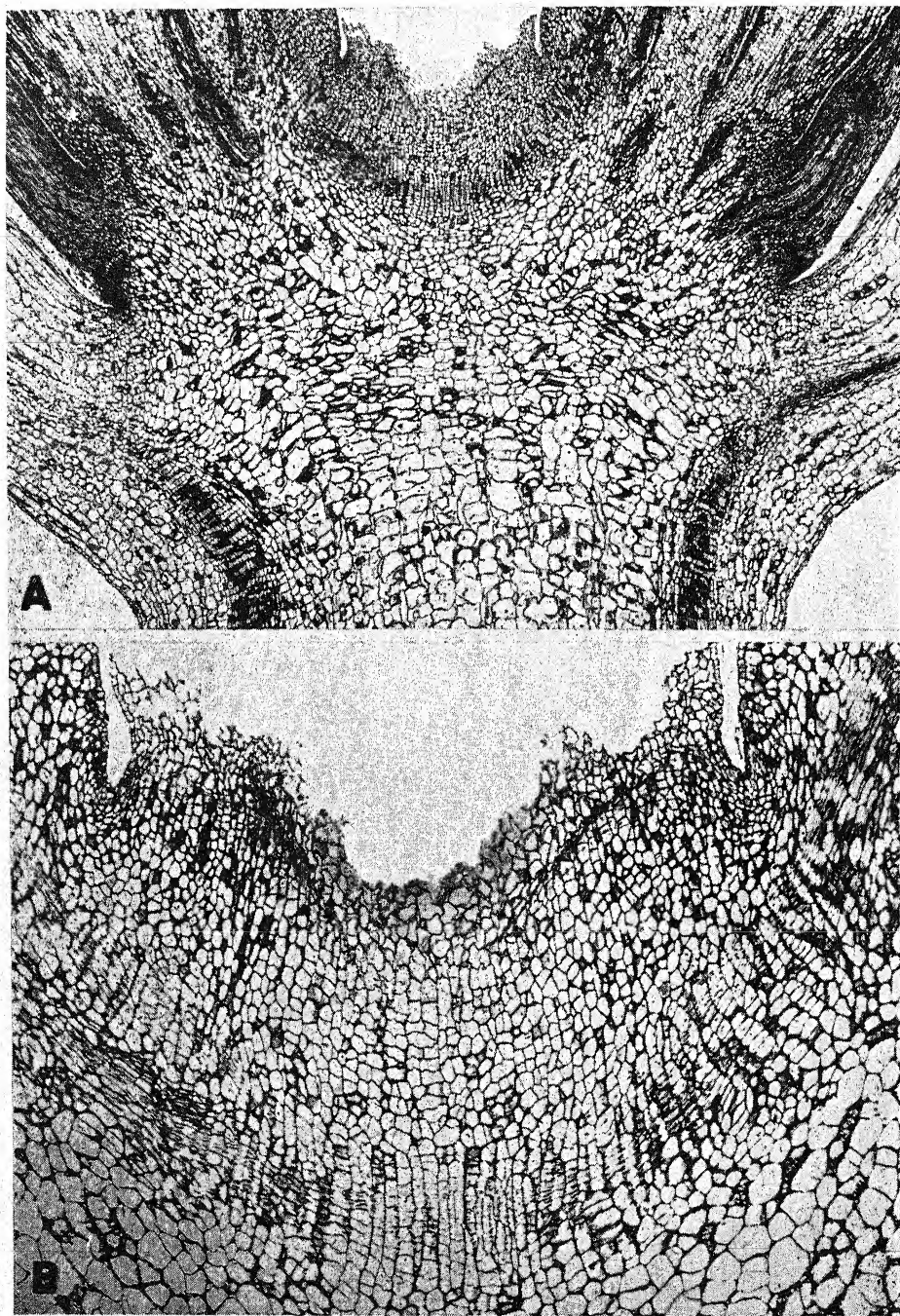


FIG. 6.—*A*, longisection of decapitated plant through abscission zone from which internode has abscised and also second meristematic zone below; *B*, details of the two zones from *A*; outer two to five layers of cells appeared to be suberized. Eighteen days after decapitation.

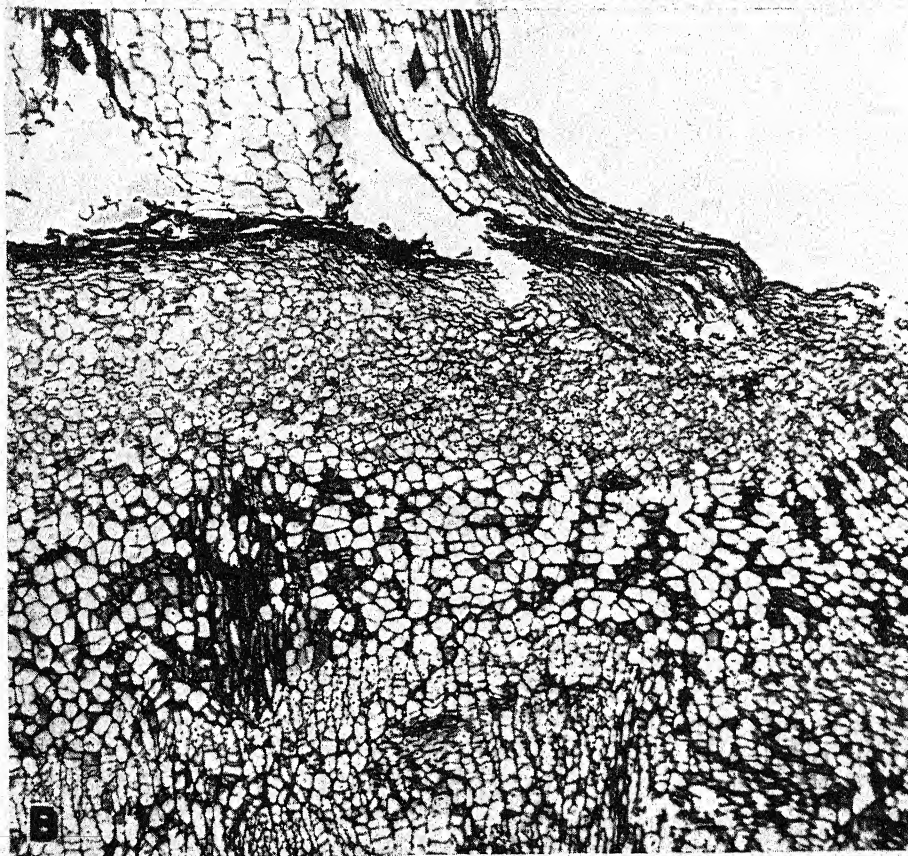
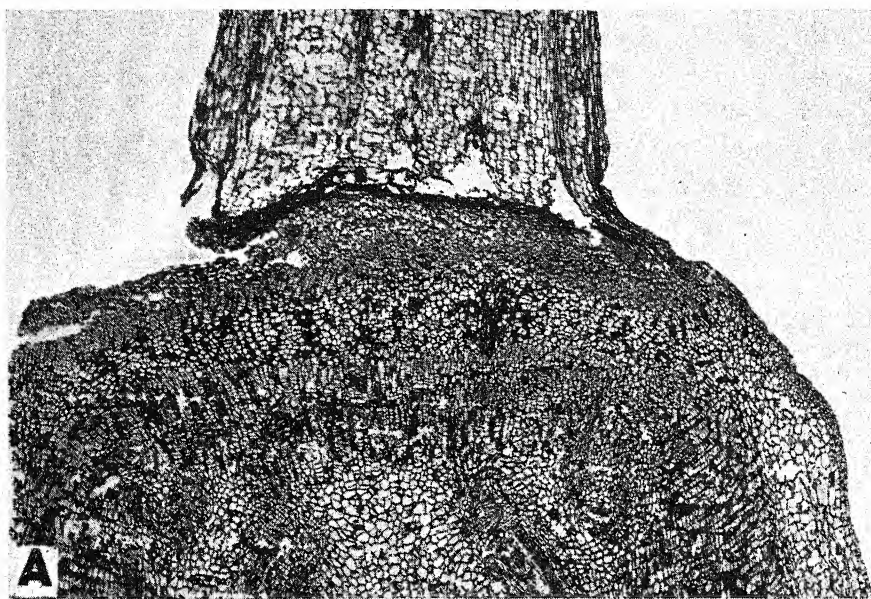


FIG. 7.—*A*, long section through intercotyledonary plane of decapitated plant, showing abscission and lower meristematic zones; *B*, details of right side of *A*; black streak at base of internode consists of crushed internode cells; abscission occurs below these cells. Twenty-four days after decapitation.

abscission layer or of the second meristematic zone associated with it. The indoleacetic acid incited no visible histological changes in the cotyledonary nodal region, and starch was stored in small quantities only (fig. 5B).

Discussion

A comparison of the two groups of decapitated plants with the untreated controls, in which abundant starch was stored, indicates that the physiological conditions have been greatly disturbed by the decapitation, whether indoleacetic acid is applied or not. The disappearance or absence of starch from the decapitated plants to which indoleacetic acid was applied is similar to the condition reported by BORTHWICK, HAMNER, and PARKER (1) for the tomato following treatment with indoleacetic acid. That an actual hydrolysis of stored starch follows the application of this and similar acids has been demonstrated by MITCHELL, KRAUS, and WHITEHEAD (7), as well as by other investigators since. Those plants which were decapitated and given no additional treatment failed to store much starch during the period of the experiments. Very small amounts of starch were observed, however, in the pith, ray, and inner cortical cells of these stems.

Not only is there an effect upon the quantity of stored starch, but the growth pattern is markedly altered through a redirection of growth from the main axis into the axillary shoots. There is also a growth difference between the two groups of decapitated plants. Those given no further treatment showed practically no additional growth in the internodes, which usually abscise within 3 weeks, although some remain attached longer. On the other hand, those decapitated and treated with indoleacetic acid showed an appreciable increase in

diameter of the internodes, with little or no shrinkage or collapse of cells. None of the internodes of this group of plants abscised during the period of 24 days, nor were evidences of an abscission zone detected in any of the longisections observed. The indoleacetic acid effectively inhibited abscission of the petioles of *M. jalapa* for the period of 24 days during which these observations were made.

Summary

1. Untreated, intact plants of *Mirabilis jalapa* showed vigorous growth of the main axis with neither abscission nor the formation of an abscission zone in internodes during the period of these experiments.

2. Plants decapitated in the upper portion of the first internode when the second internode had just started to elongate and given no additional treatment showed cessation of growth in the internodes, followed by their abscission after 2 weeks or longer. Preceding abscission, two transverse meristems developed. The upper formed across the base of the internode and developed into the zone in which abscission occurred. The lower meristem, invariably present when the upper developed, seemed to play no active role in the process of abscission. Relatively little storage starch remained in the cotyledonary nodal region of these plants at 2 weeks after decapitation.

3. Plants similarly decapitated and treated with a 2% indoleacetic acid-lanolin mixture on the cut surface showed continued growth of the internodes, complete disappearance of stored starch, and the entire absence of abscission or of an abscission zone at the bases of the internodes.

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MORPHOLOGICAL CHANGES IN PEACH SEEDLINGS FOLLOWING AFTER-RIPENING TREATMENTS OF THE SEEDS¹

H. B. TUKEY AND R. F. CARLSON

Introduction

Peach seeds (*Prunus persica* Batsch) will not germinate immediately after they reach maturity; they must first be conditioned or after-ripened. The after-ripening processes proceed most rapidly at 3°-5° C. in a moist medium, such as damp sand or peat moss. Ten to twelve weeks have been found adequate for after-ripening the types of peach seeds used by the nursery trade and in commercial horticultural enterprises.

When non-after-ripened seeds are dissected, so that the endosperm, nucellus, and integuments may be removed and the embryos placed in an environment favorable to germination, they will germinate and grow without previous after-ripening. The seedlings which develop, however, show dwarfed and anomalous growth characteristics (1, 4). The leaves are sometimes short and broad rather

than lanceolate, are crinkled along the midrib, with uneven development of halves of the blade, and with whitish patches at the margins; other leaves may be small and stipule-like. The internodes are usually shortened, and a rosette of anomalous leaves frequently appears at the apex of the epicotyledonary axis.

This paper reports the results of tests to determine the effect of different lengths of periods of after-ripening on the morphological development of the seedlings.

Material and methods

There is much variation in the behavior of peach seeds from different varieties and sources. With this in mind, pits² were secured from nineteen varieties and twelve sources,³ from three

² In this paper the term "pit" is used to denote the stony pericarp and inclosed seed, and the term "seed" is used in its strictly botanical sense.

³ The writers are indebted to the following for supplying peach seeds used in these studies: Dr. Omund Lilleland and Dr. E. L. Proebsting, Uni-

¹ Journal Article no. 627 of the New York State Agricultural Experiment Station.

seasons, totaling twenty-nine lots, as follows: Ambergem, Belle, Champion, Early Crawford, Elberta (two sources), Halehaven, Ideal, J. H. Hale, Lovell (eight sources), McAllister, Muir, Naturals (three sources), October Elberta, Polly, Salwey, Sunbeam, USSR 106, USSR 557, and Ward Late. More than 10,000 seeds were used in the course of the investigation.

sand or moist peat moss. In some treatments the entire pit was used; in others, only the seed. The material was germinated in soil in the greenhouse (68° F.) and in 100-mm. petri dishes on moist filter paper in the laboratory (68° F.). Seedlings were grown in soil in 6-inch pots in the greenhouse.

For excised embryos, the methods described by TUKEY (5) were employed,

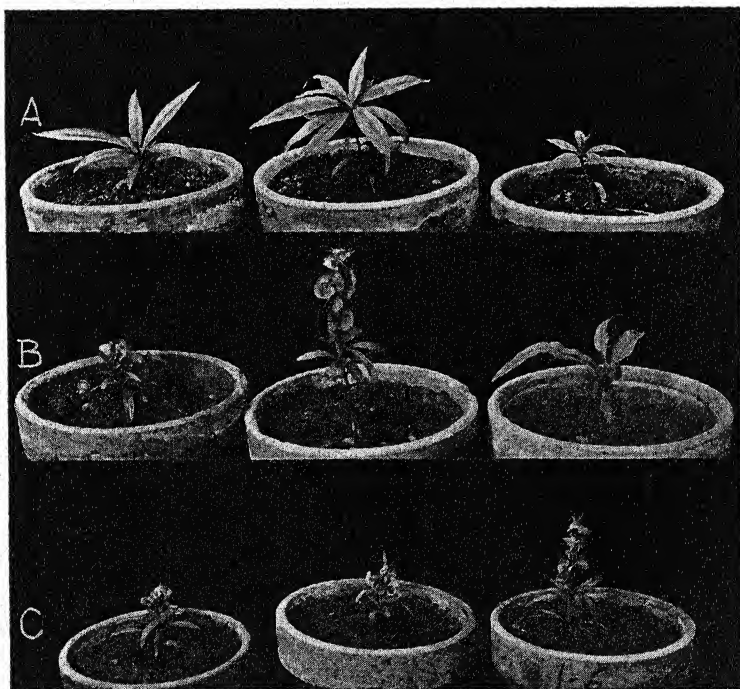


FIG. 1.—Dwarfed peach seedlings from seeds which germinated after 3–6 weeks of after-ripening. *A*, USSR 106, 3 weeks; *B*, Lovell 1, 4 weeks; *C*, Lovell 2, 6 weeks. Cf. fig. 2 for differences in varietal response following same treatment.

Conditions favorable to after-ripening were provided in a controlled refrigerator maintained at 34°–36° F., the seeds being held in containers filled with either moist

using both $\frac{1}{2}$ -oz. bottles and agar, and also filter paper in petri dishes. Most satisfactory results were secured with the agar cultures in bottles.

Results

EXPERIMENT 1: SEEDS AFTER-RIPENED 3–12 WEEKS AND PLANTED AT WEEKLY INTERVALS

Approximately 100 seeds from each of ten lots of pits were placed under condi-

versity of California, Davis, Calif.; Kelly Nursery Company, Fordsville, Ky.; Harry Malter, Greening Nursery Company, Monroe, Mich.; Dr. Stanley Johnston, South Haven, Mich.; Wm. Shaefer, Sparta, Mich.; Howard Maloney, Maloney Brothers, Dansville, N.Y.; R. L. Holmes, Jackson & Perkins Company, Newark, N.Y.; and Tennessee Nursery Company, McMinnville, Tenn.

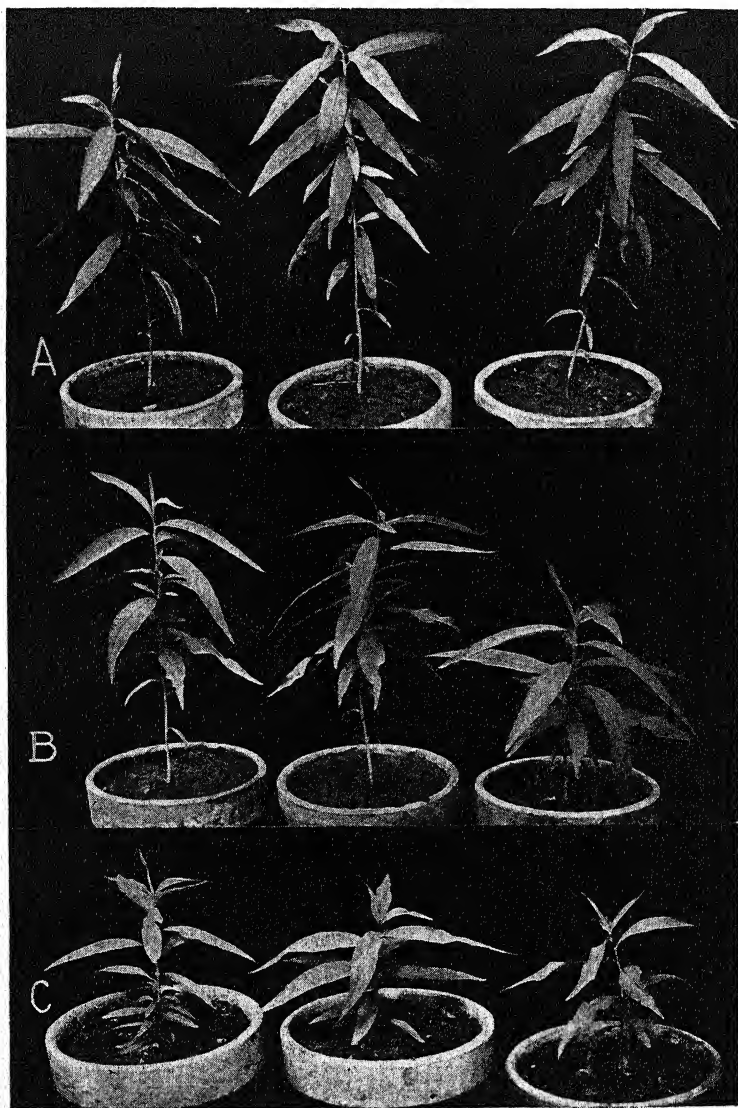


FIG. 2.—“Normal” peach seedlings from seeds which germinated after 3–6 weeks of after-ripening. A, Salwey, 3 weeks; B, McAllister, 4 weeks; C, October Elberta, 6 weeks. Cf. fig. 1 for differences in varietal response following same treatment.

tions favorable to after-ripening. Ten from each lot were removed at weekly intervals for 3-12 weeks and placed on moist filter paper in covered petri dishes. Of the seeds which germinated, representatives were planted in soil-filled pots

time. As the period lengthened, there were fewer such plants, until finally after 12 weeks there were none.

The frequency of occurrence of dwarfed and anomalous plants varied with the variety (figs. 1, 2). The variety

TABLE 1
DISTRIBUTION OF "NORMAL" AND DWARFED PEACH SEEDLINGS
FROM SEEDS AFTER-RIPENED 3-12 WEEKS

VARIETY	PLANT TYPE	WEEKS OF AFTER-RIPENING									
		3	4	5	6	7	8	9	10	11	12
Belle.....	N*	1	1	1	3
	D	1	3	2	4	0
Champion.....	N	0	2	3	2	5	4	1	1
	D	2	3	1	3	0	0	0	0
Lovell 1.....	N	1	1	4
	D	3	4	4	2	4	1	1
Lovell 2.....	N	0	1	0	0	0	0
	D	1	1	2	1	2	1
McAllister.....	N	4	8	2	2	3	2	3	3	4
	D	2	1	0	0	0	0	0	0
October Elberta.....	N	0	1	1	5	2	3	1	2	5
	D	1	1	1	0	0	0	0	0	0
Salwey.....	N	3	4	5	8	8	9	7	3
	D	0	0	0	0	0	0	0	0
USSR 106.....	N	0	1	2	3	2	4	1	2	4
	D	2	1	0	0	0	0	0	0	0
USSR 557.....	N	0	2	2	1	5
	D	1	1	1	1	1	1	0
Ward Late.....	N	1	1	1	2	1	2
	D	5	4	1	0	0

* N, normal; D, dwarfed.

and grown for 6-8 months in the green-houses.

Some of the plants were dwarfed, with anomalous leaves (fig. 1); others showed no variations from typical vigorous seedling growth of well-after-ripened seeds (fig. 2). The dwarfed and anomalous plants from any given lot were most frequent among the samplings which were after-ripened for the shortest period of

Salwey, which appears to have a relatively short after-ripening requirement, produced no dwarfed plants, even with as little as 3 weeks of after-ripening. Belle and Champion, which appear to have an intermediate after-ripening requirement, produced both dwarfed and non-dwarfed plants throughout the 6-week period of after-ripening but produced only "normal" plants thereafter.

The Lovell variety, which appears to have a relatively long after-ripening requirement, produced dwarfed plants even after 11 weeks of after-ripening.

The numbers of both dwarfed and normal plants for the different lots during

EXPERIMENT 2: EXCISED EMBRYOS OF
NON-GERMINATING SEEDS AFTER-
RIPENED 3-12 WEEKS

Some of the seeds used in the preceding experiment, although apparently

TABLE 2

DISTRIBUTION OF "NORMAL" AND DWARFED PEACH SEEDLINGS FROM EMBRYOS OF
EXCISED SEEDS WHICH FAILED TO GERMINATE AFTER
3-12 WEEKS OF AFTER-RIPENING

VARIETY	PLANT TYPE	WEEKS OF AFTER-RIPENING									
		3	4	5	6	7	8	9	10	11	12
Belle.....	N*	0	0	0	0	1	1
	D	1	1	2	1	1	1
Champion.....	N	0	0
	D	1	1
Lovell 1.....	N	0	0	0	0
	D	1	1	1	1
Lovell 2.....	N	0	0	0	0	0
	D	1	1	1	2	1
McAllister.....	N	0	1	1
	D	2	1	0	0
Muir.....	N	0	0	0	0	0	0	0	2
	D	6	1	5	4	4	3	1	4
October Elberta.....	N	1	1	1
	D	0	0	0
Salwey.....	N	(All germinated; none required excising)									
	D
USSR 106.....	N	0	0	0	0	1
	D	2	2	1	1	0
USSR 557.....	N	0	0	0	0	0	1
	D	7	1	1	2	1	1
Ward Late.....	N	0	0	0	1
	D	1	1	2	0

* N, normal; D, dwarfed.

the 12-week period are given in table 1. They show the after-ripening requirements for the production of normal plants for the other varieties not mentioned in the text.

sound, failed to germinate. Since it has been shown that embryos from non-after-ripened seeds will germinate when excised, representatives of the non-germinating seeds were excised and grown

to seedling size by the embryo-culture technique.

The results are shown in table 2. The lower number of individuals involved is due to the fact that most of the seeds germinated, leaving only a relatively few for the excising treatment. As in the previous experiment, some seedlings were dwarfed while others were typical of vigorous-

non-excised seeds following the same length of after-ripening. Thus, with variety USSR 106 only normal plants were observed from seeds which germinated after 5 weeks of after-ripening, whereas with seeds which did not germinate unless excised, the dwarfed forms appeared even after 10 weeks of after-ripening.

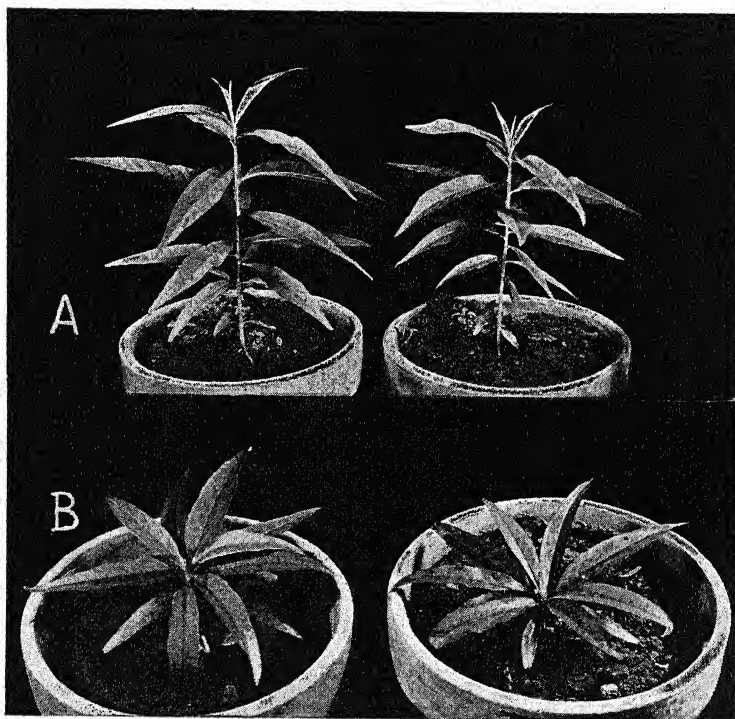


FIG. 3.—“Normal” and dwarfed seedlings from seeds of USSR 557 after-ripened for 5 weeks, showing specific after-ripening requirements for individual seeds of the same variety. *A*, normal seedlings from seeds which germinated after 5 weeks of after-ripening; *B*, dwarfed seedlings from seeds which failed to germinate until embryos were excised and cultured.

growing seedlings from well-after-ripened seeds (fig. 3). Likewise, the frequency of anomalous forms was highest with seeds which had been after-ripened for a short period; it decreased progressively as the period of after-ripening lengthened.

A comparison of tables 1 and 2 shows that the frequency of anomalous plants is higher from the excised than from the

EXPERIMENT 3: EXCISED AND NON-EXCISED SEEDS AFTER-RIPENED 1-12 WEEKS AND PLANTED AT WEEKLY INTERVALS

Six hundred seeds each of the varieties Elberta and Lovell were placed in conditions favorable to after-ripening. Fifty of each variety were removed at weekly intervals for 1-12 weeks, in each

case ten being excised and forty non-excised. Each series of 100 was then planted in soil in pots in the greenhouse.

The resulting plants were similar to those reported in experiments 1 and 2. The frequency of dwarfed forms from both excised and non-excised seeds was relatively high for the shorter periods of after-ripening and became progressively lower as the after-ripening period lengthened.

The frequency of dwarfed seedlings was again higher from the excised than from the non-excised seeds following the same number of days of after-ripening.

EXPERIMENT 4: SUCCESSIVE LOTS OF SEEDS AFTER-RIPENED 1-12 WEEKS AND THEN PLANTED SIMULTANEOUSLY

In the previous experiments, all seeds were placed in an environment favorable to after-ripening on the same day, and samples were removed at weekly intervals for 12 weeks and placed immediately in an environment favorable to germination. The question of daylength or of some other factor might conceivably have been introduced to affect the behavior of the seedlings, inasmuch as seedlings planted 12 weeks later than others were naturally subjected to a different daylength and probably to other factors. To avoid these possibilities, a test was conducted in which twelve lots of seeds were placed at successive and weekly intervals (1-12 weeks) in an environment favorable to after-ripening and were then all removed and planted on the same day. Two varieties were used, Lovell and Kentucky Naturals, and twenty-five seeds were used for each sample.

The results were similar to those from the previous experiments. Seedlings which developed from seeds after-ripened

for relatively short periods (3-6 weeks) showed dwarfed and anomalous leaf characters, whereas seedlings from seeds after-ripened for longer periods (10-12 weeks) showed no such characters.

EXPERIMENT 5: PARTIALLY AFTER-RIPENED SEED

Two lots of Lovell pits were secured from commercial sources in California. They had been exposed out-of-doors in the vicinity of Davis and so were somewhat after-ripened. When the pits were received, some in one lot had already cracked, and some seeds were germinating, the radicles being 5-10 mm. in length. The germinating seeds were separated from the non-germinating seeds in uncracked pits, and twenty of the former and thirty-five of the latter were planted in the greenhouse in soil.

All twenty of the germinating seeds developed into seedlings typical of those from well-after-ripened seed. On the other hand, only seven seedlings developed from the thirty-five non-germinating seeds, and all seven were dwarfed, with anomalous leaves.

LOSS OF ANOMALOUS CHARACTERS

The dwarfed and anomalous growths only rarely occurred elsewhere than in the epicotyledonary axis and its appendages. Occasionally a lateral shoot developed with anomalous leaves, terminating in a rosette, but this was the exception. However, new shoots arising from axillary buds showed no anomalous growth characters (figs. 4, 5). Further, when dwarfed plants were placed in a cool room (40° F.) for a month, so that they were checked in growth, and then brought into the greenhouse (65° F.), the new growth failed to show anomalous characters.

Discussion

The results from after-ripening suggest a relation to the growth patterns secured from culturing immature embryos in artificial media (6). Thus, no development may occur in peach embryos excised earlier than 51 days after full bloom when grown on 0.6% agar with 0.5% glucose and salt mixture T. Embryos excised 51 days after full bloom may show spreading and greening of the cotyledons, and small, white epicotyledonary leaves; 73 days, green cotyledons, hypocotyl 2-4

formation, stem 40-50 mm. in length, terminated by rosette of small, green stipule-like leaves, with peachlike leaves along the stem; 108 days, root and shoot development but leaves often broad and crinkled and plants dwarfed; and 118 days, root and shoot development but dwarfed plants.

The experiments reported here also suggest a relation to nutrition. Some of the anomalous growths are not unlike those secured from mineral deficiencies (2). It may be that during the after-rip-



FIG. 4.—Terminals of dwarfed seedlings, showing nature of abnormality: A, A', epicotyledonary axis terminating in rosette of anomalous leaves; B, B', normal shoots arising from axillary buds. (Note in B' the misleading appearance of "recovery" of the terminal bud from the dwarfed condition, whereas the new normal growth really arises from a lateral bud.)

mm., epicotyl 1-2 mm., terminating in a rosette of small stipule-like appendages; 80 days, roots 10-20 mm. in length, epicotyledonary axis 7-15 mm., surmounted by a rosette of white, stipule-like appendages; 87 days, vigorous root development, central axis of epicotyl 20-22 mm. in length, terminated by a rosette of green stipule-like appendages; 94 days, vigorous root development, central axis of epicotyl 25-30 mm., terminated by rosette of small, green stipule-like appendages, occasional peachlike but malformed leaves; 105 days, vigorous root

ening process certain materials are made available.

There is apparently a relation to vernalization indicated in the results, in which the subsequent performance of plants may be markedly altered by low-temperature treatment of the seeds. LYSSENKO (3) has considered the developmental processes in a plant to be made up of individual steps and stages which proceed in strict sequence. A given stage cannot proceed until the preceding stage has been completed, and different stages require different external conditions.

Vernalization and after-ripening are similar, in that they are both physiological preconditioning treatments which significantly affect subsequent behavior of the plant.

In the case of peach seedlings, the anomalous growth is confined to the epicotyledonary axis and its appendages, and new shoot growth from axillary buds only rarely shows dwarfed characters. A

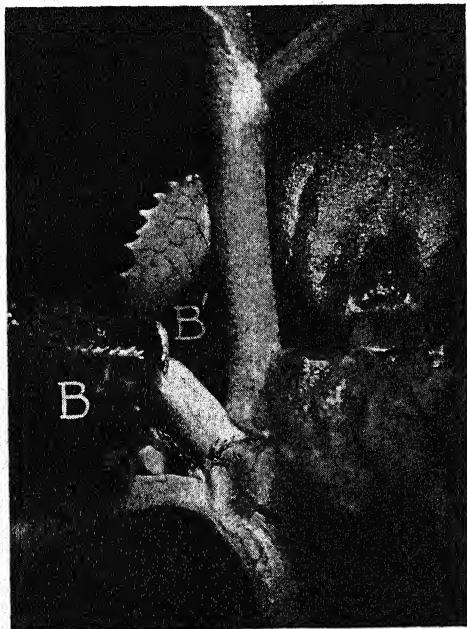


FIG. 5.—Apex of dwarfed seedlings which terminated in a rosette of anomalous leaves at B. "Normal" shoot (B') arising from axillary bud and assuming ascendancy gives the misleading appearance of "recovery" of terminal bud from dwarfed condition.

study of the epicotyl during after-ripening is being made to determine whether morphological changes accompany the process.

The importance of complete after-ripening of fruit-tree seeds in horticultural enterprises, as in the production of fruit-tree seedlings, has not been determined. However, a high percentage of dwarfed and anomalous plants has been observed among sweet cherry seedlings

which were suspected of having been subjected to insufficient after-ripening.

Summary

1. More than 10,000 peach seeds, involving nineteen varieties and twelve sources, were after-ripened at 34°–36° F. for weekly intervals ranging from 1 to 12 weeks, and then germinated and grown to seedling size at 68° F.

2. Some of the plants which developed were dwarfed, with anomalous leaves, resembling plants grown from excised embryos of non-after-ripened seeds. Such anomalous plants were most frequent among samplings which were after-ripened for the shortest period of time; they decreased progressively as the after-ripening period lengthened, until all seedlings were "normal."

3. The frequency of anomalous plants was higher from non-germinating, excised seeds than from germinating, non-excised seeds following the same length of after-ripening.

4. The frequency of anomalous plants varied with the variety.

5. Anomalous forms are not the result of photoperiod alone, inasmuch as both normal and dwarfed seedlings developed from seeds planted the same day.

6. Of two lots of pits from California, some were germinating and some not, when received. Of the germinating seeds, all developed into normal seedlings; only a few of the non-germinating ones developed, and these were all anomalous.

7. The dwarfed and anomalous growth of seedlings only rarely occurred in parts other than the epicotyledonary axis and its appendages. New shoots induced from axillary buds were free from dwarfed and anomalous characters.

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A RAPID EXTRACTION METHOD FOR FREE AUXIN AND ITS APPLICATION IN GEOTROPIC REACTIONS OF BEAN SEEDLINGS AND SUGAR-CANE NODES

J. VAN OVERBEEK, G. DÁVILA OLIVO, AND ELBA M. SANTIAGO DE VÁZQUEZ

Introduction

In a study of the growth and development of sugar cane (18), it became desirable to determine its auxin content. Various methods for the determination of the free auxin of plant tissues have been described in the literature (19). Among the most suitable methods for the extraction of free auxin are those in which heat is employed in order to prevent, during the extraction, liberation of auxin from bound forms or precursors. Heat is alleged to destroy an enzyme system responsible for this liberation or activation (5, 13). The immersion of sugar-cane material in boiling water for a brief period stopped the continued yield of auxin during the process of prolonged extraction.

A complication arose, however, when it became evident that as a result of the heating a substance was liberated from the cane material which has an inhibitory effect on the curvature of the *Avena* test. This substance is ether-soluble and is therefore extracted together with the auxin. It thus became necessary to find a more suitable method of extraction, which does not involve heating of the

plant material. Since previous research (for literature, see 19) has shown that the liberation of free auxin from inactive forms is a slow process, it seemed that free auxin, exclusive of that activated in the course of the extraction, could be removed from the plant by an extraction period of short duration. With this in mind, a study was made of the rate at which auxin was removed from fresh sugar-cane material during extraction with ether. The curves obtained indicated that large amounts of auxin are removed during the first half hour of the extraction, and relatively little later on.

To find convincing evidence that this first gush represented free auxin, a series of rapid extractions were made from the upper and lower sides of geotropically stimulated bean seedlings. It has been known for some time that geotropically stimulated seedlings yield more free auxin on the lower than on the upper side, as determined by the diffusion technique (for literature, see 21). For this reason it is assumed that the lower side contains more free auxin than the upper. By using a rapid-extraction technique, a difference in auxin content between the two sides of

the stimulated bean seedlings was found which corresponded exactly with that obtained previously by means of the diffusion method. It seems, therefore, that the rapid-extraction technique yields a valid quantitative estimate on the free auxin of fresh material.

General methods

The sugar-cane variety most often used in these experiments was P.O.J. 2878, a variety developed in Java (P.O.J. is an abbreviation for Proefstation Oost Java—Experiment Station, East Java). It ranks among the more important cane varieties in Puerto Rico (7). The nodes were often used for trials on extraction techniques because they contain more auxin than the rest of the stalk (fig. 1). For the experiments with bean seedlings, Bountiful variety stringless beans (Kilgore) were employed. The seeds were germinated in sand in a darkroom and the stems used about 5 days after planting.

Before the auxin was extracted, the fresh material was cut into pieces about $5 \times 2 \times 2$ mm. and frozen by solid CO_2 whenever this was available. As an extraction solvent, purified diethyl ether was employed. The extract was reduced to a few cubic centimeters and poured on top of a known amount of hot agar in a vial 22 mm. in diameter and 48 mm. in height. The use of this larger size of vial constitutes a simplification in technique over that previously employed (16), in that it allowed the ether to be poured directly from the flask onto the agar. With the smaller vials previously used, the ether had to be carefully added, drop by drop, by means of a pipette to the hot agar in order to prevent spilling.

When the ether has evaporated, the auxin is automatically dissolved in the agar. This was subsequently poured into molds, and the resultant agar plates were

then divided into sixteen small blocks ($3.5 \times 3.5 \times 1.4$ mm.), which in turn were applied to the decapitated plants in the *Avena* test.

The general principles of the *Avena* test (21) were followed, with the following modifications:

1. Owing to the even temperature and humidity at Mayagüez, as compared with more northerly climates, it was found that no special equipment for their control was necessary. Records of

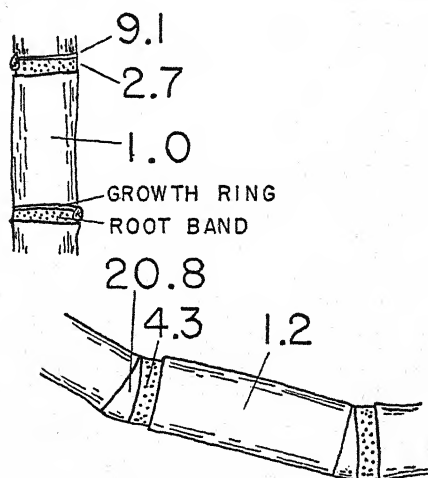


FIG. 1.—Distribution of free auxin (indoleacetic acid equivalents γ /kg. fresh weight) as determined by rapid extraction ($2 \times \frac{1}{2}$ hr.) with ether, in vertical and horizontal sugar cane (cane in horizontal position for 4 days). Plant material frozen in dry ice prior to extraction. The leaf is attached just below root band.

the thermograph showed that in the darkroom the average temperature was about 78°F. and that it rarely varied more than 2° during any 24-hour period. The humidity, although more variable than the temperature, could as a rule be maintained at 80% during the actual tests. This is the most desirable humidity for the *Avena* test when the plants are grown in sand.

2. Very satisfactory results (20) could

be obtained by planting the hulled and germinated oat seedlings (Victory variety) in moist sand in zinc boxes ($20 \times 3 \times 3$ cm.) instead of in the usual glass holders. The seedlings were planted more closely than is customary in the test in which glass holders are used. This allowed a certain degree of selection.

3. In earlier experiments, an interval of $1\frac{1}{2}$ hours between the first and second decapitation was used, as is customary in the "standard" *Avena* test (21); later this period was extended to 3 hours, as suggested by SCHNEIDER and WENT (9).

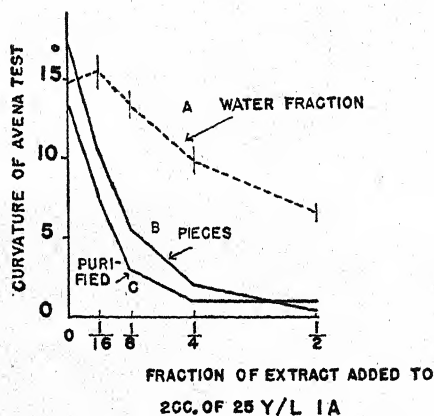


FIG. 2.—Depressing effect on auxin curvatures of *Avena* test by extracts from basal nodes of sugar cane boiled 10 minutes in water (quantity " $\frac{1}{2}$ " on abscissa corresponds to 50 gm. fresh weight of nodes). A, ether extract of water fraction of boiled nodes. B, ether extract of residue of boiled nodes. C, same as B except that fats and waxes were removed from extract by refluxing with petroleum ether; ether extraction lasted over-night. Vertical lines of A indicate magnitude of standard error of *Avena* test ($n = 20-29$).

The concentration curves obtained with synthetic indoleacetic acid under the conditions just described were similar to those obtained under the conditions of the standard *Avena* test in Pasadena, California. Here, as well as there, two general types of curves were obtained: (a) those showing a direct proportionality

between auxin concentration and curvature, and (b) those inflected at the lower end and therefore showing a threshold (15). No periodic changes in sensitivity of the *Avena* test to a given amount of indoleacetic acid has been found so far. A more detailed account of the test and the auxin-extraction technique under tropical conditions has been given elsewhere (20). The magnitude of the standard error of the *Avena* tests may be judged from figure 2.

Results

SUBSTANCES ANTAGONISTIC TO AUXIN

Heating of plant tissues, followed by extraction with ether, has been recommended (5) as a method of extracting free auxin. When the nodes of sugar cane were thus treated, unsatisfactory results were obtained. Auxin yields were low and the results were not reproducible. In addition, if auxin was obtained it was found impossible to get reasonable concentration curves. In an attempt to investigate this unusual behavior, it was decided to add the extracts of boiled cane nodes to agar containing a known amount of indoleacetic acid. It was found that, as more of the extract was added to a given amount of indoleacetic acid, it depressed more the action of this auxin in the *Avena* test. In one test, for example, when agar containing indoleacetic acid in a concentration of 50 γ per liter was tested by itself, it caused a curvature of 15.4° in the *Avena* test. When, however, an amount of extract equivalent to 8 gm. fresh weight of cane nodes, which were submerged in boiling water for 3 minutes prior to ether extraction, was added to 2 cc. of this auxin agar, the curvature was reduced to 9.8° ; and when extract of 33 gm. fresh

weight of boiled cane node was added, the action of the indoleacetic acid was almost entirely blocked.

Some extracts have been reported (11) which cause a "positive chemotropic" curvature in the *Avena* test; that is, a curvature opposite in direction from that caused by auxins. Extracts of boiled sugar-cane material when taken up in plain agar showed no evidence of inducing such positive chemotropic curvatures in the *Avena* test. Neither did preparations of the inhibitor cause bud inhibition when applied to the apical surface of vertical seed-pieces of cane.

In an attempt to separate the inhibitory substances from the auxins, the water fraction was filtered from the remaining pieces of cane immediately after boiling. Both fractions were extracted over-night with ether, and various portions of these extracts were added to 2 cc. of agar which contained 25 γ indoleacetic acid per liter. The results show that both the water and the solid fractions contain the inhibitory substance. However, the solid fraction yielded larger amounts (cf. curves A and B in fig. 2).

In further fractionation attempts, the ether extract of boiled cane nodes was evaporated to dryness, then water was added, which in turn was extracted twice with petroleum ether by boiling under reflux for one-half hour. The petroleum ether removed the lipoidal material, including the waxy substances, but the greater part of the inhibitory activity remained in the water fraction (fraction insoluble in petroleum ether), as shown by curve C in figure 2. In this respect the inhibitor resembles auxin. The fact that an extract from which the waxy substances have been removed still shows most of the inhibitory activity eliminates the possibility that these waxy substances in some mechanical way prevented the

auxin from entering the *Avena* test plant and that this would account for the depressing effects of the boiled cane extracts.

RATE OF AUXIN EXTRACTION AS A FUNCTION OF TIME

The rate at which auxin is extracted from sugar-cane nodes varies considerably during the course of the extraction (20). This is again demonstrated in figure 3, which shows that during the first half hour of the extraction a relatively large amount of auxin is released

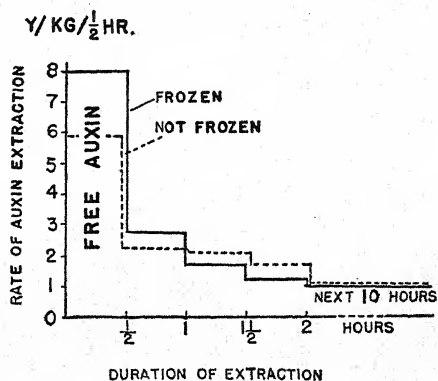


FIG. 3.—Rate of auxin yield from fresh sugar-cane nodes during extraction with ether. High initial yield is considered to represent principally the free auxin of tissue at beginning of extraction.

from the tissue, while later considerably smaller amounts are yielded during similar periods of time. The technique consisted of cutting the nodes into small pieces and placing them in Erlenmeyer flasks containing purified ether. Some samples were frozen in solid CO_2 prior to being extracted. During the extraction the flasks were occasionally shaken, and after one-half hour the ether was decanted. The residue was washed twice with fresh ether and then extracted for another period of one-half hour. This process was repeated until four extractions of one-half hour each were made. Then

a fifth extract was made which lasted for 10 additional hours, and a sixth which lasted for 10 additional days. A number of experiments were conducted during the course of a year, the results of which agreed with the one presented previously (20) and with table 1 and figure 3 of the present paper.

Preliminary freezing in dry ice promoted the liberation of auxin during the first period. Crushing of the frozen material in a mortar did not materially in-

RAPID EXTRACTION METHOD AND AUX- IN DISTRIBUTION IN GEOTROPICALLY STIMULATED BEAN SEEDLINGS

It should be possible to test the theory that the gush during the first half hour of the extraction represents free auxin, as one would conclude from the shape of the curve of figure 3. It has been conclusively demonstrated that more auxin can be obtained by diffusion from the lower side of geotropically stimulated seedlings than from the upper side. This

TABLE 1

AMOUNTS OF AUXIN (INDOLEACETIC-ACID EQUIVALENTS γ /KG. FRESH WEIGHT) EXTRACTED
FROM SUGAR-CANE NODES DURING PROLONGED EXTRACTION WITH ETHER.
ENTIRE EXPERIMENT RUN IN DUPLICATE

	NUMBER AND SEQUENCE OF EXTRACTIONS					
	1	2	3	4	5	6
Duration of extraction (hours) . .	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	10	240
Material frozen in dry ice. . . .	$\begin{cases} 7.8 \\ 7.6 \end{cases}$	$\begin{cases} 2.4 \\ 2.9 \end{cases}$	$\begin{cases} 2.6 \\ 1.3 \end{cases}$	$\begin{cases} 0.9 \\ 0.8 \end{cases}$	$\begin{cases} 24.0 (1.2)^* \\ 14.4 (0.7) \end{cases}$	$\begin{cases} 72 (0.15) \\ 98 (0.20) \end{cases}$
Material frozen in dry ice and ground.	$\begin{cases} 9.2 \\ 7.2 \end{cases}$	$\begin{cases} 1.8 \\ 3.6 \end{cases}$	$\begin{cases} 2.4 \\ 1.2 \end{cases}$	$\begin{cases} 2.3 \\ 1.0 \end{cases}$	$\begin{cases} 24.0 (1.2) \\ 22.8 (1.1) \end{cases}$	$\begin{cases} 91 (0.19) \\ 90 (0.19) \end{cases}$
Material neither frozen nor ground.	$\begin{cases} 5.0 \\ 6.8 \end{cases}$	$\begin{cases} 1.9 \\ 2.6 \end{cases}$	$\begin{cases} 2.2 \\ 2.1 \end{cases}$	$\begin{cases} 2.4 \\ 1.0 \end{cases}$	$\begin{cases} 20.4 (1.0) \\ \dots\dots\dots \end{cases}$	$\begin{cases} 100 (0.21) \\ \dots\dots\dots \end{cases}$

* Figures in parentheses are rate of extraction per $\frac{1}{2}$ hour.

crease the auxin yield over that of material only frozen.

The gush of auxin released during the first hour suggests that this represents most, if not all, the "free auxin," while the auxin which is liberated gradually represents auxin released from inactive or bound forms during the extraction. This release of auxin at a slow rate by the tissue may continue for many months in sugar cane (20), as well as in other tissues (5, 13). It cannot be due to insufficient removal of auxin during the first extractions, since freezing and grinding of the tissue does not alter the nature of the slow extraction (5, 13).

by its very nature is free auxin. Since more free auxin diffuses out of the lower side, it may be assumed that this side contains more free auxin than the upper. It was therefore decided to extract, by the rapid method, the upper and lower halves of the hypocotyls of geotropically stimulated etiolated bean seedlings. The seedlings were cut off at the base and pinned on a horizontal board by means of cork disks. For 24 hours they remained on this board in a horizontal position, in the dark and in a saturated atmosphere. At the end of this period the cotyledons were removed and the hypocotyl split lengthwise into upper and lower portions.

The portions were then weighed and dropped in separate Erlenmeyer flasks containing purified diethyl ether. In some experiments the material was frozen in dry ice prior to extraction. Three extractions were made, each lasting one-half hour. As a rule, a fourth extraction lasting 24 hours was made. All operations took place in the darkroom, with the ex-

that the gush of auxin of figure 3 represents principally free auxin.

Table 2 also shows the results of *prolonged* extraction of the upper and lower halves of geotropically stimulated bean hypocotyls. It is obvious that the difference between the two sides, which was so striking during the early part of the extraction, has disappeared. This is entirely

TABLE 2
AUXIN EXTRACTION FROM UPPER (U) AND LOWER (L) HALVES OF GEOTROPICALLY STIMULATED HYPOCOTYLS OF ETIOLATED BEAN SEEDLINGS

TEST	FIRST EXTRACTIONS $3 \times \frac{1}{2}$ HOUR				NEXT EXTRACTION 24 HOURS			
	$\gamma/\text{kg.}/\frac{1}{2}$ hours		Relative		$\gamma/\text{kg.}/24$ hours		Relative	
	U	L	U	L	U	L	U	L
1.....	6.4	12.0	35	65	5.6	4.3	57	43
2.....	8.0	11.7	41	59				
3.....	4.1	7.7	35	65	3.9	2.8	58	42
4.....	2.2	7.9	22	78	5.8	4.9	54	46
5.....	4.9	7.6	39	61	3.7	2.8	57	43
6*	3.0	9.7	24	76				
7*	3.0	10.5	27	73	14.0	12.0	54	46
8*	7.0	9.9	41	59	5.8	5.3	52	48
Average (extraction)...	4.8	9.6	33	67	6.5	5.4	55	45
Average (diffusion) of data for seedlings in literature (21).....			34	66				
Rate of extraction $\gamma/\text{kg.}/\frac{1}{2}$ hour.....	2.4				0.13			

* Material frozen in dry ice prior to extraction.

ception of the decanting and the readdition of ether.

The results, summarized in table 2, show clearly that by means of rapid extraction 67% of the total auxin is obtained from the lower side and 33% from the upper. This is in striking agreement with the results obtained by diffusion, which show (21, p. 165) that from seedlings, on the average, 66% is obtained from the lower and 34% from the upper side. This strongly supports the view

in agreement with the assumption that the auxin which is slowly released during the later part of the extraction represents auxin activated or released from a precursor or a bound form during the extraction itself. As compared with the first rapid extraction, the ratio between the upper and lower halves seems to be reversed. On the average, 45% of the auxin extracted during the 24 hours following the first rapid extraction was recovered from the lower side,

while 55% was obtained from the upper.

GEO-INDUCED AUXIN INCREASE IN NODES OF SUGAR CANE

The nodes show marked response to geotropic stimulation. A few days after

wedge-shaped body measuring 1 cm. or more at the lower end. A study was undertaken of the auxin relations in these nodes. (For the anatomy of sugar cane, see 1.) The canes were geotropically stimulated by forcing part of the culms of a stool into a horizontal position by

TABLE 3

AUXIN OBTAINED BY RAPID EXTRACTION FROM SUGAR-CANE STEMS (NON-CULTIVATED JAPANESE VARIETY) IN VERTICAL (V) OR HORIZONTAL (H) POSITIONS. MATERIAL NOT FROZEN PRIOR TO ETHER EXTRACTION (1½ HOURS). DATA EXPRESSED IN INDOLEACETIC-ACID EQUIVALENTS (γ/KG.). UPPER OF EACH PAIR OF FIGURES IN H COLUMNS REPRESENTS AUXIN CONCENTRATION OF UPPER HALF OF GEOTROPICALLY STIMULATED STEM; LOWER, THAT OF LOWER HALF

	TIME AND POSITION														
	1 day		2 days		3 days		4 days		5 days						
	V	H	V	H	V	H	V	H	V	H					
Growth ring. . .	1.6	2.9 2.8	2.9	3.3	2.9 3.5	3.2	0.8	3.7 3.7	3.7	4.8	8.4 10.6	9.5	6.0	12.2 9.6	10.9
Average vertical.	4.0									5.2	11.6 11.6	11.6	6.6	12.0 11.6	11.8
Difference H-V.	1.3		-0.1		2.9		5.6		5.1						
Root band.	1.8	1.8 1.7	1.8	3.3	2.6 2.3	2.5	1.2	1.7 2.1	1.9	3.4	6.0 4.8	5.4	5.0	6.0 7.2	6.6
Average vertical.	3.2									3.2	4.2 4.6	4.4	4.8	5.0 4.8	4.9
Difference H-V.	0.0		-0.8		0.7		1.6		0.9						
Middle of inter- node.	1.1	0.6 1.3	1.0	2.6	2.3 2.3	2.3	1.2	1.1 1.2	1.2	2.8	3.0 2.8	2.9	4.6	2.7 2.2	2.5
Average vertical.	2.4									2.6	1.8 2.0	1.9	3.4	2.0 1.8	1.9
Difference H-V.	-0.1		-0.3		0.0		-0.3		-1.8						

the canes have been placed horizontally, the growth ring (the intercalary zone, shown in figure 1, and which is only a few millimeters thick when the cane is in the vertical position) will grow into a

means of ropes. The entire plant remained intact—no leaves were removed and the stem retained its connection with the root system.

After the canes had been in a horizon-

tal position for a certain length of time, which varied between 1 and 7 days, these canes, together with the remaining vertical ones from the same stool, were harvested. The growth rings, root bands, and a section of the middle part of the internodes were removed from the younger portion of the stalk, excepting the youngest apical portion. In the horizon-

prior to extraction. There is a striking increase in auxin concentration of the growth ring after canes have been in a horizontal position for a few days. This effect was evident in all tests, without exception. The effect is less obvious in the root band and may be absent in the internode.

The unilateral distribution of auxin,

TABLE 4

AUXIN OBTAINED BY RAPID EXTRACTION FROM VERTICAL (V) AND HORIZONTAL (H) SUGAR-CANE STEMS (VARIETY P.O.J. 2878). EXPERIMENTAL CONDITIONS AS IN TABLE 3, BUT MATERIAL FROZEN IN DRY ICE PRIOR TO RAPID EXTRACTION ($2 \times \frac{1}{2}$ HOUR) WITH ETHER

	TIME AND POSITION					
	3 days		4 days		7 days	
	V	H	V	H	V	H
Growth ring	17.0	$\left. \begin{array}{l} 40.7 \\ 26.6 \end{array} \right\} 33.7$	9.1	$\left. \begin{array}{l} 26.4 \\ 15.2 \end{array} \right\} 20.8$	10.0	$\left. \begin{array}{l} 19.5 \\ 22.6 \end{array} \right\} 21.1$
Average V	12.0					
Average H	25.2					
Root band	3.8	5.8	2.7	4.3	3.3	9.3
Average V	3.3					
Average H	6.5					
Middle of internode .	0.5	1.8	1.0	1.2
Average V	0.8					
Average H	1.5					

tal canes these sections were also subdivided into upper and lower portions with respect to their position during geotropic stimulation. These various tissues were cut into small pieces, as previously described, and either directly extracted for a short time (table 3) or frozen in dry ice prior to the rapid extraction.

The results are summarized in tables 3 and 4, while figure 1 shows the results of a single rather typical experiment in which the tissue was frozen in dry ice

which is so characteristic for geotropically stimulated seedlings, seems to be entirely absent in sugar cane. Neither the growth ring, the root band, nor the internode showed any evidence of a higher auxin concentration on the lower side. Because of the excellent results obtained with rapid extraction in bean seedlings, it is unlikely that the method is responsible for this failure to find the seedling type of lateral auxin distribution in sugar-cane nodes.

CHEMICAL NATURE OF EXTRACTED AUXIN IN BEANS AND SUGAR CANE

A number of experiments were conducted to determine whether the auxin obtained by extraction from the various materials used in the present investigation is of the type of indoleacetic acid or of auxin-a or auxin-b. The principal tests

TABLE 5

RELATIVE STABILITY TO ACID AND ALKALI OF AUXINS FROM SUGAR CANE AND BEAN SEEDLINGS. FIGURES IN HORIZONTAL ROWS ARE COMPARABLE AND INDICATE AUXIN CONTENT OF SOLUTIONS OF EQUAL VOLUME IN DEGREES OF CURVATURE IN AVENA TEST

SOURCE OF AUXIN	REACTION OF SOLUTIONS DURING AUTOCLAVING		
	Neutral	NaOH 0.01 N	H ₂ SO ₄ 0.01 N
Synthetic indoleacetic acid.....	16.0	12.1	0.8
Purified, from sugar-cane nodes.....	8.9 5.2	6.8 10.8	0.0 0.8
	Neutral	KOH 1 N	H ₂ SO ₄ 1 N
Rapid extraction from etiolated bean hypocotyls.....	10.3 12.1 12.8	13.3 10.5 12.0	1.1 +1.5 0.5
Same, but by prolonged, following rapid, extraction...	1.7 0.0 5.5	9.4 5.6 13.9	1.4 +0.5 0.0

were acid- and alkali-stability determinations; but for sugar cane, additional determinations of the diffusion coefficient were made. The results of the latter have been reported previously (20). All determinations without exception indicated that the auxin extracted is of the type of indoleacetic acid.

The auxin extracted from sugar cane was purified by refluxing with petroleum ether for two periods of 30 minutes each.

This treatment removed the lipoidal material but did not decrease the auxin content of the extracts. The purified extracts were made acid (to 0.01 N H₂SO₄), alkaline (to 0.01 N NaOH), or left neutral, and then autoclaved for 15-30 minutes at 15 lb. pressure. A control solution of synthetic indoleacetic acid was run for comparison. After autoclaving, the extracts were neutralized, made equal in volume, and tested for their auxin content. The same procedure was followed for the auxin extracted from bean seedlings, except that the purification with petroleum ether was omitted and stronger acid and alkali (1 N) were used. Table 5 shows that the auxin extracted from all materials is stable to alkali and unstable to acid. In this respect it resembles indoleacetic acid and not auxin-a, which is stable to acid, or auxin-b, which is destroyed both by acid and alkali (6). With respect to acid and alkali stability, there is no difference between auxin obtained by rapid extraction and that obtained by a subsequent prolonged extraction (table 5).

Discussion

This paper has dealt with two main points, the development of a practical method for the extraction of free auxin and a comparison between the auxin relations in geotropic reactions of seedlings and of grass nodes.

For extracting the free auxin from plant tissues, without extracting in addition an appreciable amount of auxin which is set free during the course of the extraction, there are three possible methods: (a) heating the plant material prior to extraction, (b) extraction at low temperature, (c) rapid extraction. Heating the plant material was shown to be undesirable because it caused the release of substances shown to be antagonistic to

auxin and therefore interfering with the *Avena* test. Extraction at low temperature was investigated on a previous occasion (20), when it was found that although low temperature (-5° C.) slows down the rate at which auxin is released during prolonged extraction, it nevertheless remains of considerable magnitude; and at the end of 3 months the same total amount was released by extraction at 24° C. as during -5° C. However, in combination with the rapid extraction technique this low temperature extraction may become useful.

The rapid extraction technique for free auxin makes use of the fact that liberation of auxin during prolonged extraction proceeds at a slow rate (13, 20). Especially when the extraction is preceded by a breaking-up of the cells of the plant tissue by means of dry ice, this method is simple and reliable. Results obtained by the diffusion technique, which unquestionably tests for free auxin exclusively, could be duplicated with the rapid extraction technique.

Techniques in which the auxin is rapidly extracted from plant tissues are by no means new. In the earliest extraction techniques, extraction was of short duration (12). They were, however, abandoned when it was found that exhaustive extraction was not obtained. Since a slow process of auxin activation (or perhaps a release of free auxin from a bound form) continues during the ether extraction, there is reason again to favor rapid extraction.¹

More auxin can be extracted from the lower than from the upper side of hori-

zontal bean seedlings by the rapid method, and this is evidence for the correctness of the assumption that largely free auxin is extracted by this method. The evidence became even more convincing when a comparison between the ratio of the yields of upper and lower halves showed that these ratios were the same in the data obtained by the diffusion technique and by the rapid-extraction technique.

There appears to be a fundamental difference in geotropic behavior between seedlings and grass nodes. Seedlings do not increase their rate of growth when stimulated geotropically when they are rotated on the horizontal shaft of a clinostat (2, 8); the nodes of grass seedlings do increase their rate of growth (4). The total amount of auxin produced by geotropically stimulated seedlings is not increased more than that of vertical seedlings (3), while in grass nodes there is considerable increase. In seedlings, more free auxin is contained in the lower than in the upper part of geotropically stimulated organs, while in grass nodes this effect is either very weak (10, 17) or totally absent, as seems to be the case with sugar cane.

There is also another point of difference which is of importance in this respect. In all seedlings there is marked response to applied auxin. In grass nodes, by contrast, auxin does not seem to cause a growth response. UYLDERT (14) reported not the slightest action of auxin on the growth of grass nodes. Auxin applications on sugar cane will promote the development of roots from the root band, and will inhibit the lateral buds (18, 20), but in no case has any auxin-induced increase in growth of the nodes per se been found. This indicates that a factor other than auxin is the controlling agent in the growth and geotropic reaction of

¹ After this manuscript had been completed, an article (WENT, F. W., Amer. Jour. Bot. 31:597-618. 1944) appeared which reported that from the stem tips of tomato plants more auxin is extracted during the first hour than during subsequent hours. This is in complete agreement with the results obtained here with sugar cane.

the sugar-cane node, and that the CHOLODNY-WENT theory (21), which seems to explain so well the geotropism of seedlings, does not hold for the geotropism of the nodes of sugar cane.

Summary

1. When tissue of sugar-cane nodes is submerged for a few minutes in boiling water, a substance is released which suppresses the action of indoleacetic acid in the *Avena* test (fig. 2). The substance is soluble in water and diethyl ether but not in petroleum ether. It is therefore extracted together with auxin, and for this reason heating of such plant material—and perhaps of plant material in general—prior to ether extraction is an undesirable step in auxin extraction technique.

2. In the course of ether extraction, there is during the first half hour a gush of auxin (fig. 3), but soon the rate slows down to a mere trickle, which continues for many months (20).

3. When the auxin of the upper and lower halves of horizontal hypocotyls of bean seedlings is extracted, using only the auxin yielded rapidly during the early part of the extraction, 33% is obtained from the upper side and 67% from the lower (table 2). This is precisely the average auxin distribution found for free auxin as determined by the diffusion method (21).

4. When the auxin of the upper and lower halves of horizontal hypocotyls of bean seedlings is extracted, using only the auxin yielded slowly during the later part of the extraction, no such unequal yields are obtained (table 2). Apparently the auxin yielded during the early part

of the extraction is the free auxin of the plant tissues.

5. A rapid-extraction technique for the extraction of free auxin consists in freezing the plant tissue in dry ice (crushing is unnecessary); extracting it in Erlenmeyer flasks in ether for two or three periods of $\frac{1}{2}$ hour each at room temperature; taking the extract up in a known amount of agar and using it on the *Avena* test (plants grown in sand instead of the usual glass holders).

6. When the free auxin content of horizontal stems of sugar cane is compared with stems in the vertical position, after a few days in the horizontal position the auxin concentration in the meristematic region of the node (the growth ring) increases 100% and more (fig. 1; tables 3, 4). This increase is less marked in the root band and is absent in the middle portion of the internodes.

7. The unilateral distribution of free auxin so striking in horizontal bean seedlings is totally absent in sugar cane. In further contrast to bean seedlings, no growth response to applied auxin—other than promotion of root growth and bud inhibition—was found in sugar cane.

8. The auxin extracted from etiolated bean hypocotyls and from sugar cane appears to be of the type of indoleacetic acid rather than auxin-a or auxin-b, in respect to stability to hot alkali and lability to hot acid. Both the auxin yielded during the early part of the extraction and that yielded during the prolonged later part belong to the indoleacetic-acid type.

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FLORAL MORPHOLOGY OF CHRYSOTHAMNUS NAUSEOSUS SPECIOSUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 567

EDNA SNOW

Introduction

Considerable work has been done on the morphology of the Compositae (2, 3, 4, 5, 6, 10, 13, 14, 15, 17, 18, 19, 20, 22, 23) but very little on the dominant composite plants of the west. HALL and CLEMENTS (11) have given attention to the taxonomy, origin, and relationship of the genera *Artemisia* and *Chrysanthemum*. DIETTERT (6) made an extensive study of *Artemisia tridentata* Nutt. in

which he describes flower and seed development and development of leaf and stem. The present work was undertaken to extend the information on the floral development, gametogenesis, and vascular anatomy of one of the more abundant species of *Chrysanthemum*, *C. nauseosus speciosus* (Nutt.) Hall & Clements.

The genus *Chrysanthemum* is divided by HALL and CLEMENTS (11) into four sections, twelve species, and forty sub-

species. *C. nauseosus speciosus* is included in the section *Nauseosi*, the only one in which the twigs are densely pannose-tomentose. The group is characterized by a dense coating of long, weak hairs, more or less impregnated with a resinous substance. *C. nauseosus* exhibits the greatest variety of form.

C. nauseosus speciosus is a shrub 2-6 feet high, broad and rounded, usually blooming through September and October, sometimes even up to December. The rounded flower heads, usually consisting of five yellow disk flowers, are massed in such great numbers that the patches of color have given the plant the common name of goldenbush. It is also known in the Great Basin area as rabbitbrush, because rabbits utilize the thickets as a place of refuge.

This plant is found chiefly in the Great Basin region, although it occurs to some extent in all the western states. It occupies large areas of desert or semidesert country, frequently in pure stands, often along washes, on plains, and on mountain slopes. The species is deep-rooted and unfitted to compete successfully with the grasses in the surface soil. Under ecologically favorable conditions it is replaced by sagebrush or wheatgrass, but becomes re-established when such cover is broken by destructive grazing.

As a potentially important economic plant, it was investigated by HALL and CLEMENTS (11), who found rubber in the cortex and medullary rays of all twelve varieties examined. They estimated that the amount of rubber present averages about 2.83% but in individual plants may be as high as 6.57%. HALL and LONG (12) estimated that 150,000 tons of wild rubber are available in the roots and stems of this species, but the scattered and isolated location of the plants would make extraction and processing

almost prohibitive, except in case of extreme need.

MATERIAL AND METHOD.—The material used in this study was collected at Provo, Utah, beginning in March, 1943, and continuing each week until October 6 of the same year, and from August 18 through November 2, 1944.

Sectioning is difficult, since the bracts are covered with small clubshaped hairs which have considerable resinous substance within the cells. Resin ducts are regularly associated with the bundles and continue into the floral tube, although they do not occur with the bundles of the rest of the flower.

Formalin-acetic acid-alcohol killing and fixing agent was used exclusively because of its rapid penetration of the resinous tissues. The fixed buds were dehydrated by the tertiary butyl-alcohol method, imbedded in paraffin, sectioned, and stained in a modification of Fleming's triple stain. All sections were cut at 10 μ , with the exception of a few longisections for the study of megasporogenesis, which were cut at 12 or 15 μ . Cross and longisections were made of all the samples collected. All drawings, with the exception of figures 55 and 56, were made with a camera lucida.

Observations

FLORAL DEVELOPMENT

The leaf buds of *C. nauseosus speciosus* appear early in March, but the flower buds do not appear until August. Development of the buds and flowers is relatively rapid. There are no over-winter buds, and the whole process of bud formation up to fertilization occurs in a period of about 6 weeks. The collections of September 4 were the first in which the archesporial cell of the ovule had developed. At this time the microsporogenous cells were undergoing reductional divi-

sions. The pollen grains were mature about September 17, and the megagametophyte by October 6.

There are usually five disk flowers in a head, each in the axil of a bract. Ray flowers are absent. The appearance of the individual flowers is essentially acropetal, the lowest one first. The second differentiates very close to it, the third and fourth at about the same time, and the fifth somewhat later.

No evidence of flower primordia appeared until July 21. At that time there was a broadening of the stem tip, with no further change until about August 17, when the flower bud primordia began to appear. Each individual flower appears as a blunt protuberance in the axil of a bract on the broadened axis. Growth is more rapid in circumference than in length, thus broadening the primordium. Zonal growth occurs at the periphery of the protuberance. At the outer edge of this zonal portion the corolla differentiates first, followed by the stamens, then the pappus, and finally the carpels (figs. 4-7). The order of development of *Chrysothamnus* is similar to that of *Aster* and *Solidago* as reported by MARTIN (17), but differs from certain others as reported by COULTER and CHAMBERLAIN (5) and MERRELL (18), in which the order of differentiation is corolla, stamens, carpels, and finally pappus.

Large nectaries appear about the same time as the archesporial cell and develop almost simultaneously with the ovule. They are located at the base of the stamens adjacent to the style of the pistil.

The corolla lobes curve inward at an early stage. Their marginal cells interlock, bringing about a fusion of the lobes (fig. 12). The corolla remains sealed until forced open by elongation of the stamens (fig. 56), as also occurs in *Artemisia tridentata* Nutt. reported by DIETERT (6).

VASCULAR ANATOMY

The stele of the stem consists of five collateral bundles (fig. 13). At the tip of the stem five fibrovascular bundles are diverged from the stele (figs. 14, 15). Each of these is related to a single flower, of which there are five. At the base of each flower the vascular trace is similar in structure to the vascular bundle in the stem, of which it is a continuation.

Just above this level in each flower there diverge from the solid stele two vascular bundles on the side farthest away from the central axis of the stem (fig. 14). At a slightly higher level three more bundles (figs. 15, 55a-b) are diverged.

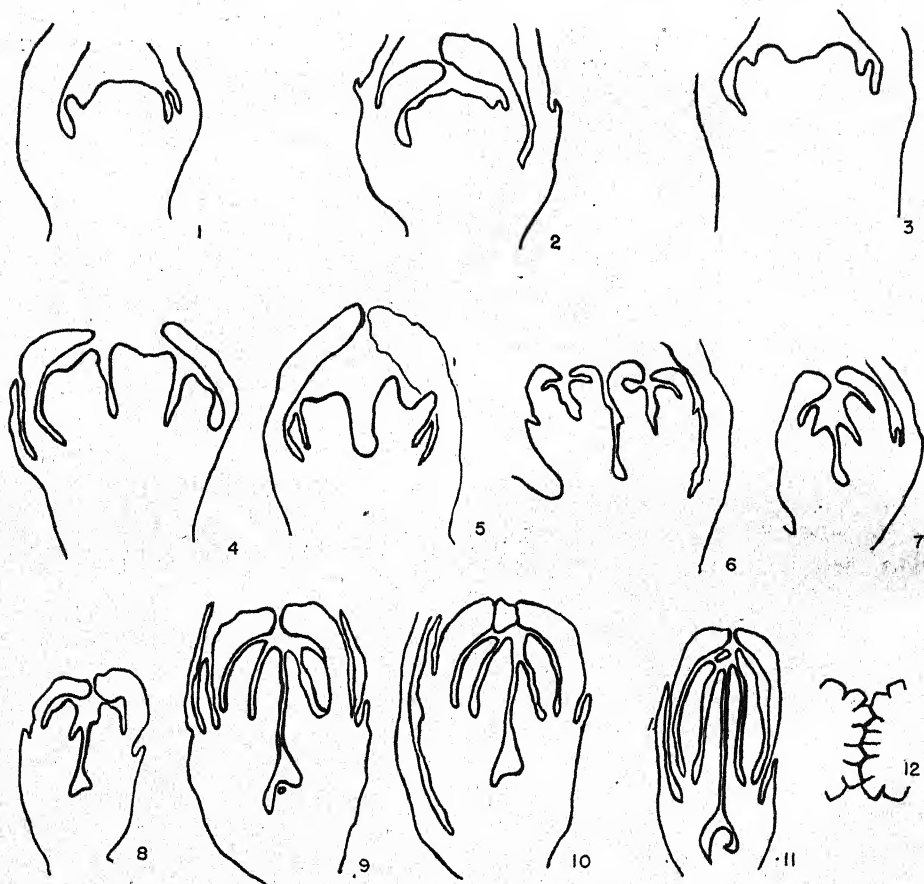
These five bundles, equal in number to the corolla lobes, extend upward through the floral tube (figs. 16, 29) to the point of divergence of the stamen, where from each a single bundle diverges into each stamen (figs. 28, 29). This occurs at about the level where the pappus arises. There is no vascular supply to the pappus. The main course of the bundles is continued upward through the corolla, but instead of being opposite to the middle lines of the lobes—as in some flowers—they alternate with them to the point of their divergence. Here each is then separated into two equal branches, each of which traverses the near margins of two adjacent lobes (figs. 31, 32).

The five major vascular bundles may be numbered in a clockwise direction, number 2 being opposite the main axis of the stem. Numbers 4 and 5 diverged first from the central stele; the other three somewhat higher.

A single bundle supplies the ovule. This is a continuation of two bundles which arise as divergences from numbers 1 and 3 of the three major bundles just described. They are distinct in the funiculus but extend as a single bundle

through the raphe and curve over the chalaza of the very large ovule, extending down the single integument. The two styler bundles diverge from bundles 2 and 5 just above the top of the ovule, the former at a slightly lower level than the

the corolla was given by BROWN (1). *Chrysothamnus* is similar to the description given by him. The corolla of the primitive Compositae has a vascular supply of fifteen bundles—ten lateral, and five median (14, 15). The median vein of



FIGS. 1-12.—Figs. 1-5, section of young flowering branch, showing beginning of heads. Figs. 6-10, early stages in development of flowers. Fig. 11, young flower showing relation of parts at archesporial cell stage. Fig. 12, portion of two corolla lobes, showing interlocking of marginal cells.

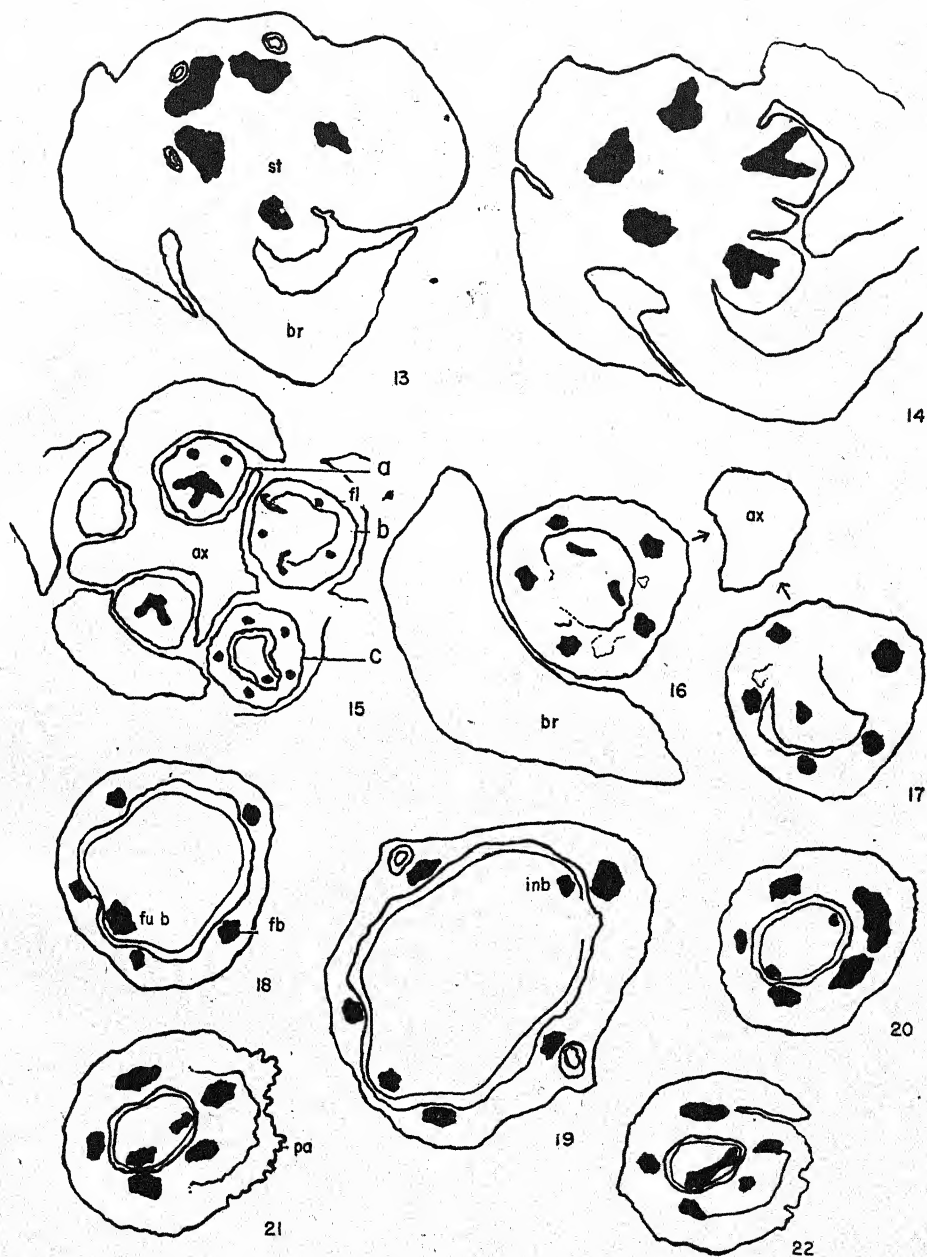
latter. These two bundles maintain their meristematic condition for a longer time than does the single bundle.

According to KOCH (14) the earliest mention of the venation of the corolla was by GREW in 1682, although the position of the veins was not discussed. The first clear description of the venation of

the petals is not present in *C. nauseosus speciosus*, there having been a reduction from this primitive condition to five bundles.

MICROSPOROGENESIS

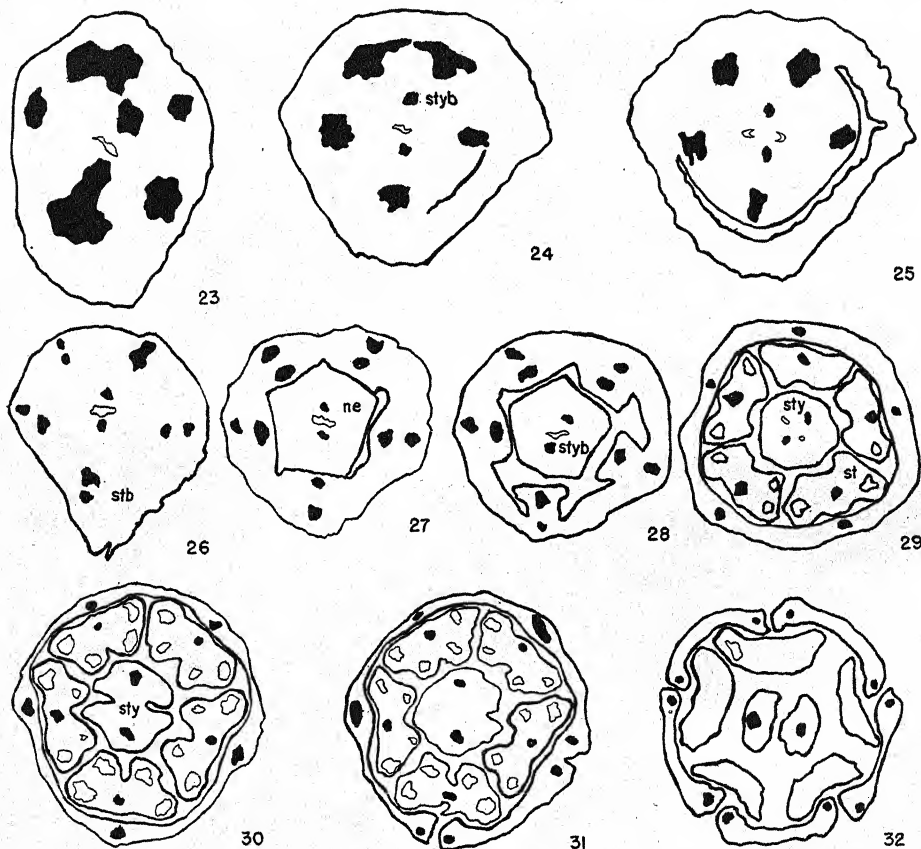
The five stamens diverge immediately above the divergence of the corolla. The



FIGS. 13-22.—Transections at different levels of flowering branch, tracing course of vascular system upward into individual flower: Figs. 13-15, tip of stem axis showing divergence into individual flowers. Fig. 16, individual flower, showing five major veins and bract. Figs. 17, 18, five bundles of floral tube and vein of funiculus. Figs. 19, 20, five bundles of floral tube and veins of funiculus and integument. Figs. 21, 22, section near top of ovule, showing curving of vein over chalaza; pappus being cut off.

young stamen soon becomes distinctly four-lobed in cross-section, each lobe representing a microsporangium. In each corner of the anther a single large hypodermal cell differentiates as the archesporial cell, which soon divides periclinally and gives rise to a single layer

undergoes periclinal divisions to form two layers. These later develop as a two-layered wall. The inner layer matures as a tapetum and consists of large, elongated cells, densely granular and usually having two or four nuclei (fig. 39A). These cells remain intact until after the



FIGS. 23-32.—Figs. 23-25, stilar bundles. Figs. 26-28, divergence of stamen bundles. Figs. 29, 30, stamen, stilar, and petal bundles. Figs. 31, 32, divergence of petal bundles to adjacent lobes. (ax, axis; brt, bract; fl, flower; fb, floral tube bundle; int, integument; inb, integument bundle; nec, nectary; pb, petal bundle; rd, resin duct; st, stamen; stb, stamen bundle; sty, style; styb, stilar bundle.)

of primary parietal cells and one layer of primary sporogenous cells. The primary sporogenous cell undergoes several successive anticlinal divisions, thus forming a group of four or five microspore mother cells.

Meanwhile the primary parietal cell

meiotic divisions have occurred; then they become digested, disintegrated, and absorbed by the developing spores. A similar condition was found in *Silphium* by MERRELL (18).

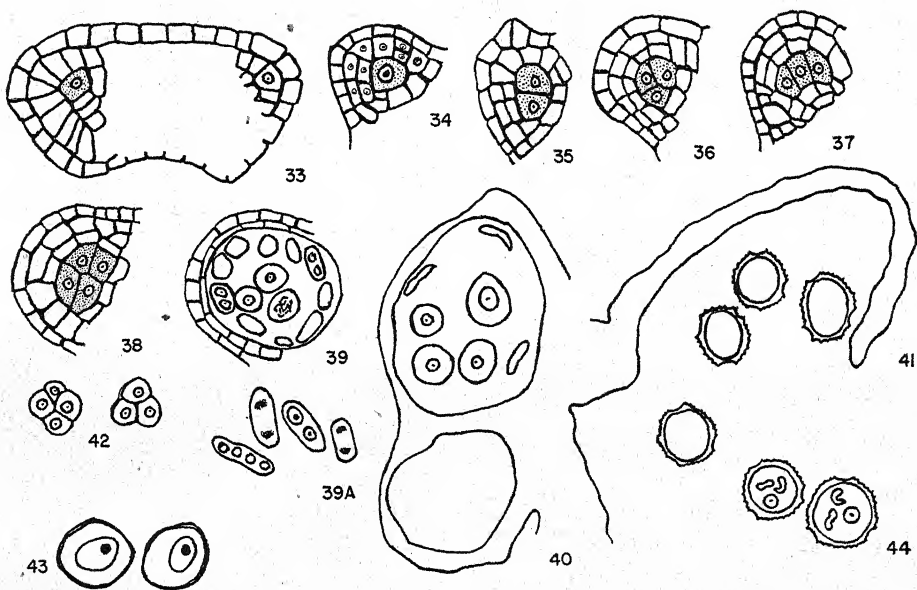
The microspore mother cells undergo meiosis (fig. 39), and the microspores

(fig. 43) are formed in tetrads (fig. 42). The tetrads split apart soon afterward, and after enlargement of the spore the nucleus divides to form a generative and a tube nucleus. The generative nucleus divides to form two spherical sperm nuclei which later elongate (fig. 44).

The mature microgametophyte in the anther is essentially spherical, having three furrows, and has a spinous wall

into the ovule instead of ending abruptly just below the edge of the funiculus. MERRELL states, "Whatever may be the condition in other Compositae, the ovule of *Silphium* as shown by its origin and its bundle relations is the termination of the floral axis, that is, the ovule is cauline."

Within the nucellar tissue the single hypodermal archesporial cell appears rather early and is easily recognized by



FIGS. 33-44.—Stages in development of anther: Figs. 33-38, sporogenous cells, primary parietal and tapetal layers. Fig. 39, pollen sac at microspore mother cell stage. Fig. 39A, cells of inner tapetal layer, showing two or four nuclei. Fig. 40, pollen sacs at microspore stage. Fig. 41, one-half mature anther, showing one pollen sac. Fig. 42, microspores, tetrad stage. Fig. 43, microspores. Fig. 44, microgametophyte at time of shedding.

(fig. 44). At this stage the sperm nuclei are elongated, are slightly crescent- to spindle-shaped, and lie free in the cytoplasm of the pollen grain.

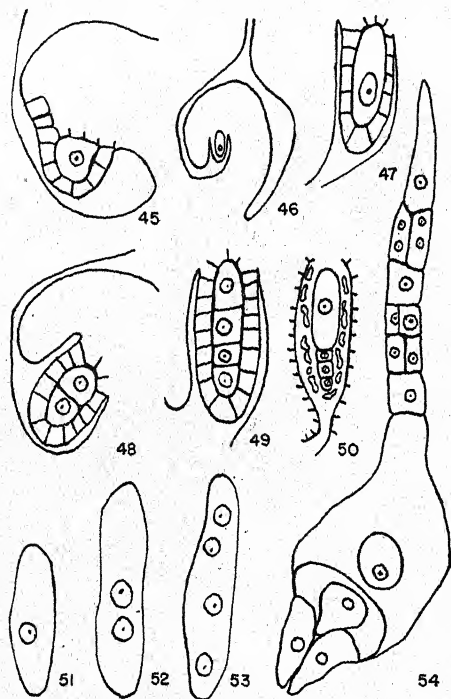
MEGASPOROGENESIS

The nucellus arises from the placenta at the tip of the axis and appears to be cauline. In the ray flower of *Silphium* (18) the axial bundle extends directly

its large size, granular appearance, and greater affinity for stains (figs. 45-47). The archesporial cell enlarges rapidly without dividing and becomes directly the megaspore mother cell. The elongated spore is covered by a single layer of nucellus. It follows the usual pattern of meiosis, forming a linear tetrad (figs. 48, 49). Three megaspores, usually those nearest the micropylar end, disintegrate

(fig. 50). The remaining one increases rapidly in size, as does the ovule, which soon fills the ovary cavity (fig. 51).

The functional megaspore undergoes three successive divisions (figs. 51-54), as has been reported previously for other Compositae (4, 5, 6, 14), forming at first a gametophyte of the eight-nucleate type. Wall formation occurs, dividing it into



FIGS. 45-54.—Stages in development of megagametophyte: Fig. 45, archesporial cell stage. Figs. 46-49, development of megaspores. Fig. 50, disintegrating megaspores (high power). Fig. 51, functional megaspore. Figs. 52, 53, development of megagametophyte. Fig. 54, mature megagametophyte showing synergids, egg, polar nucleus, and nine antipodal cells.

seven distinct cells. Subsequent divisions of the antipodals occur. The mature megagametophyte occupies about one-half of the entire length of the ovule. The lower two-thirds of the sac is narrow and elongated, but the central region of the upper one-third increases in breadth.

None of the material examined showed fusion of the polar nuclei, which came to rest near the egg cell, but it apparently occurred early. The egg cell is pear-shaped, attenuated toward the micropylar end. The synergids, which are in close contact with the egg, are rather slender, with attenuated ends which extend into the micropyle. As already stated, the antipodal cells often divide, so that a variable number is formed. These differ in size and arrangement. Megagametophytes having six to ten antipodal cells were found, and some of these cells had more than one nucleus (fig. 55). The antipodals are arranged in a more or less linear row, although in several cases two cells lie side by side. Often such cells have been cut off in planes at right angles to each other. The antipodal cell farthest from the micropyle is usually the largest. It elongates rapidly, often occupying about one-third of the space of the antipodal tissue. These results agree closely with those of CHAMBERLAIN (2) and OPPERMAN (19), but are in contrast with those of MARTIN (17), who found no walls for these cells, and not more than four antipodal cells, and never in a single longitudinal row. The antipodal tissue persists very late in the development of the embryo.

GUIGNARD (8, 9) was one of the first to describe the increased development of the antipodals. He found them persisting and increasing in number up to ten uninucleate cells in *Conyza ambigua*. COULTER and CHAMBERLAIN (5) described as a common feature of the Compositae the enlargement of the distal antipodal cell and its penetration into the chalazal tissue. The nucellar cells adjacent to the antipodals are elongated and loosely connected. The antipodal cells are equal to—or exceed—the rest of the megagametophyte in length.

The nucellar tissue surrounding the two-thirds of the gametophyte nearest the micropyle begins disintegration almost simultaneously with that of the three nonfunctional megasporos, and few of its cells remain when the four-nucleate

The cells of the integument are narrowly columnar, compact, densely granular, stain darker, resemble a secondary nutritive layer, and form the "epithelial" layer characteristic of the Compositae as described by HOWE (13).

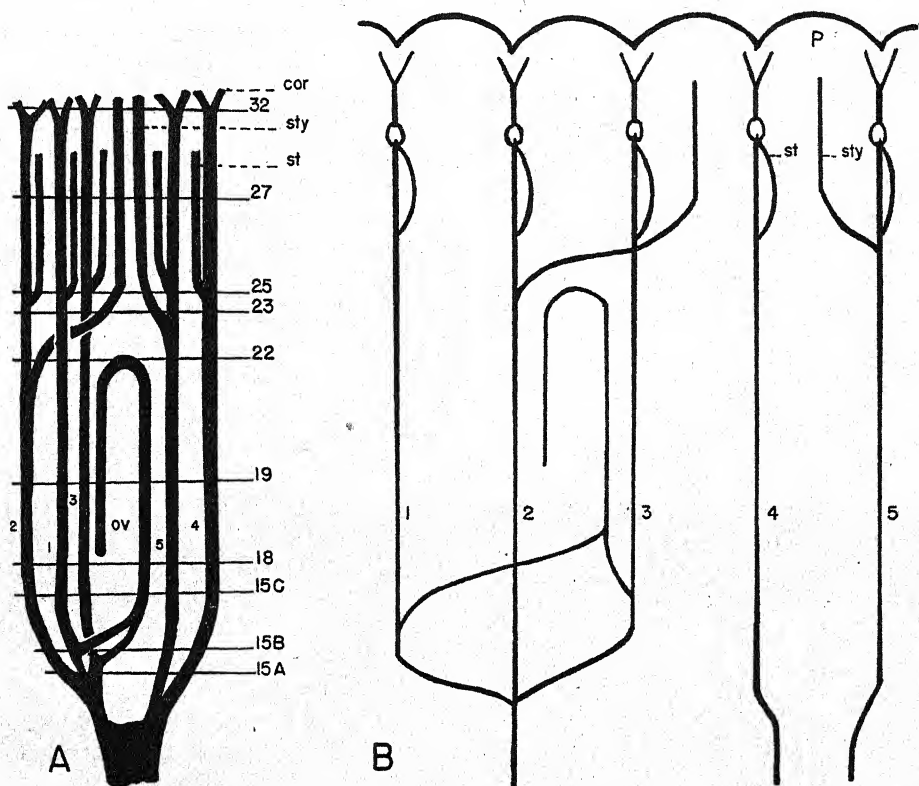


FIG. 55.—Diagram showing A, vascular anatomy of flower in longisection in three dimensions; B, same, in two dimensions.

stage of the megagametophyte is reached. When the megagametophyte is mature this nucellar tissue has disappeared. Following the disintegration of the nucellar tissue, one or two inner layers of cells of the integument closely surround and are in contact with the megagametophyte, except its antipodal portion—which projects into the tissue of the ovule near the chalazal region. This tissue becomes digested and absorbed by the gametophyte.

Discussion

The development of the flowers and the vascular anatomy of *Chrysanthamnus nauseosus speciosus* extends, and agrees somewhat with, the observations made by SMALL (22), CHAMBERLAIN (2, 3), MARTIN (17), KOCH (14, 15), MERRELL (18), OPPERMAN (19), BROWN (1), WARMKE (23), and GUSTAFSSON (10). In order of floral development it is like that of *Silphium*, in which the pappus appears

before the pistil, but unlike *Aster*, *Solidago*, and *Erigeron*, in which the pistil is the last structure to differentiate.

KOCH (14) describes the vascular system of the composite flower and divides it into four types. The fourth or discoid type which she describes is typical of *C. nauseosus speciosus*, and according to her it is the advanced type. In this type

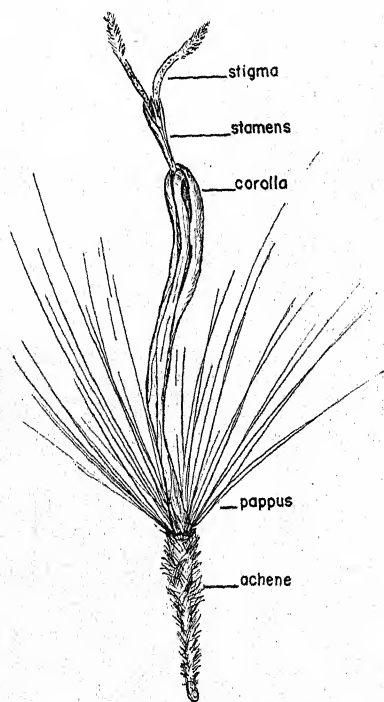


FIG. 56.—Old flower just prior to loss of corolla, stamens, and stigma.

there is an absence of midveins in the corolla lobes of the tubular flower. The presence of veins bordering the margins of the lobes is explained on the basis of the fusion of the lateral bundles of primitive Compositae, and the loss of the median bundle. The five fused lateral bundles of the floral tube divide at the sinuses and border the corresponding lobes.

BROWN (1) describes this condition

and states that the Compositae agree in two points: "1. Valvular aestivation, or the margins of segments dehiscing like capsule valves. This may be found in other families. 2. The nerves of the corolla are equal to the lobes, but instead of being opposite and passing through their axes as in other plants, they alternate with them, each divides into two equal branches at the top of the tube."

The absence of pith in the stele as it diverges into the individual flowers is rather an unusual condition. So far as the writer is aware, this feature is not mentioned in the literature on the Compositae.

The styler bundles, diverging at slightly different levels from bundles 2 and then 5 (fig. 55A-B), bear out the work of SMALL (22). He states, "BROWN gives the orientation of the styler bundles correctly as antero-posterior and the ovarial bundles as lateral."

The vascular anatomy of a plant is especially important in explaining epigyny. The nature of the floral tube has been described by WILSON and JUST (24), and also by EAMES as reported by DOUGLAS (7). They state that there is abundant proof from the course of the bundles that the inferior ovary represents adnation in its extreme form. The vascular anatomy of *C. nauseosus speciosus* shows that the floral tube is not an invaginated axis but may be composed of undiverged basal parts of the flower. Certainly there is in *Chrysothamnus*, as well as in other Compositae, considerable zonal growth of all parts up to the top of the ovary (figs. 9, 10), and of the petals and stamen for a considerable distance above that level (fig. 11).

There seems to be general agreement that the Compositae are characterized in general by having male gametes existing as naked nuclei in the pollen grain,

elongated and curved, or irregular in shape (PODDUBNAJA [20] as reported by DIETTERT [6]).

One notable feature in *Chrysothamnus* is the relatively extreme size of the ovule. It grows rapidly after the archesporial cell stage. A representative set of measurements showed the gametophyte to be about 1.4 mm. and the ovule about 3 mm. in length, the latter equaling about one-fourth the full length of the flower. In my material no ovules were found with more than a single archesporial cell.

The antipodal cells vary in number, size, and position, as well as in number of nuclei. The usual number of three antipodals was not seen in this species. Discussions of the number and character of the antipodal cells in the Compositae have been given by COULTER and CHAMBERLAIN (5), SMALL (22), and SCHNARF (21). The enlargement and extension into the chalazal tissue is common and corresponds with the description of *Artemisia* reported by DIETTERT (6). Most investigators agree that the haustorial type of antipodals is concerned with the nutrition of the megagametophyte and that they aid either in digestion, or in conduction (or both) of substances to the gametophyte (8, 16, 20).

At the time the megagametophyte is developing, disintegration of some of the cells of the ovule occurs. These cells lie immediately interior to the epidermis of the ovule, principally in the chalazal region. The epidermis of the ovule remains intact and becomes thickened at the time the megagametophyte is mature, but the walls of the two layers of cells interior to it have been broken down. This seems to be a common occurrence in all the sections studied, but no mention of this disintegration has been found in the literature.

Summary

1. *Chrysothamnus nauseosus speciosus* (Nutt.) Hall & Clements is a potentially important economic shrub of western United States. It is 2-6 feet high, and is a subclimax dominant of the sagebrush-wheatgrass association.

2. It blossoms through September and October. The yellow flower heads are rounded, usually consisting of five disk flowers, with no ray flowers present.

3. The flower bud primordia appear during the latter part of August, and fertilization occurs the early part of October. The floral organs develop in the sequence: corolla, stamens, pappus, and pistil.

4. The marginal cells of the corolla lobes curve inward at an early stage, interlock, and bring about fusion of the lobes. Later the elongation of the stamens forces the flower open.

5. The fibrovascular bundles are equal in number to the corolla lobes but are alternate with their median lines rather than opposite. The styler bundles are antero-posterior, and the ovule bundle is lateral.

6. The mature microgametophyte at the time of shedding has three nuclei, a tube nucleus, and two elongated naked sperms.

7. A single archesporial cell was found in each ovule. It gives rise to four megaspores. The chalazal megaspore is the functional one.

8. The nucellus surrounding the mature megaspore mother cell and the four spores consists of one layer of cells. There is a single massive integument and a single large ovule. The inner layer of the integument is in contact with the megagametophyte and appears tapetal in nature.

9. The mature megagametophyte con-

sists of two synergids, an egg cell, two polar nuclei which fuse early, and a variable number of antipodals which show much diversity in their size, number, and arrangement, but are mainly linear. The number of antipodal cells varies from six to ten, each having from one to several nuclei. The cells are persistent and usually become haustorial in nature, penetrating the chalazal region and causing dis-

integration of the cells surrounding the region.

The writer acknowledges with sincere thanks the advice and help given by members of the Department of Botany of the University of Chicago during the course of this investigation.

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RELATION OF TEMPERATURE TO THE SELECTIVE HERBICIDAL EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID

P. C. MARTH¹ AND F. F. DAVIS²

Introduction

In the course of previously reported investigations with 2,4-dichlorophenoxyacetic acid as a selective herbicide (2, 3, 6, 9), it was observed, under conditions in the field, that spray applications made in late fall and early winter were much slower in inducing plant responses than those applied during warmer periods. Herbicidal effects of 2,4-dichlorophenoxyacetic acid are to some extent dependent on the continuance of plant growth for a period of time following treatment. It is therefore to be expected that temperature may influence the rate of response and govern, to some extent, the time required to kill plants by this means. Furthermore, the effect of temperature is pronounced with applications of growth regulators other than 2,4-dichlorophenoxyacetic acid, and with plant-growth responses other than those of a herbicidal nature (1, 4, 5, 7).

The present experiments were conducted in the vicinity of Beltsville, Maryland. They were undertaken to determine the effects of temperature on the responses of some weeds to treatment with 2,4-dichlorophenoxyacetic acid or with its sodium salt. Some plants were kept at relatively low temperatures, others at medium, and others at high temperatures. The plants were subse-

quently treated and grown under these respective temperature conditions. Other plants were treated and grown for several weeks at a low temperature and then transferred to a higher one so as to induce more rapid vegetative growth. In addition, field applications of both the acid and its sodium salt were made in December on dormant plants of *Plantago lanceolata* in soil which was frozen to a depth of 2 inches. Subsequently, separate lots of the treated and the untreated plants were brought into the greenhouse at biweekly intervals, and their growth under temperature conditions (65° F.)³ favorable to vigorous vegetative growth was observed.

METHODS.—In general, aqueous sprays containing 0, 500, 1000, and 1500 p.p.m. of 2,4-dichlorophenoxyacetic acid were applied to twenty-four or more plants of each species growing at the various temperatures. In preparing the sprays, the acid was first dissolved in sufficient Carbowax 1500 (8) to give a final concentration of 1.0% of the wax. Sprays containing 1000 p.p.m. of the sodium salt of the acid were prepared by dissolving the salt directly in a measured volume of water containing a small quantity of laundry soap to serve as a spreader. Carbowax was not used in the preparation of salt solutions. All spray applications were made with a 1-quart capacity compressed-air sprayer operating at 60-lb. pressure. A measured volume of spray was applied to each lot of plants, so that approximately the same coverage was obtained in each instance.

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³ All temperatures throughout this paper are Fahrenheit.

Results

In an experiment initiated on November 16 and 17, several hundred 1-year-old plants of *Barbarea verna* (early winter cress) and *Plantago lanceolata* (narrow-leaved plantain) were transplanted from the field to 4-inch clay pots. The plants were then allowed to grow in a greenhouse at 65°–75°, for a period of 27 days in the case of *Barbarea* and for 43 days in the case of *Plantago*, during which time both species became well estab-

the time of treatment. At intervals of 2–4 days thereafter, records were made of the number of leaves which subsequently turned yellow, the degree of epinasty, or the death of the plants in the different treatments. In the case of *B. verna* the rate of loss of green foliage color followed closely the rate of dying of the plants under the different spray treatments and temperature exposures, whereas with the other species the number of days required for complete killing

TABLE 1

EFFECT OF GREENHOUSE TEMPERATURE ON RELATIVE RATE OF KILLING OF *BARBAREA VERNA* FOLLOWING AQUEOUS SPRAY APPLICATION CONTAINING 500-, 1000-, AND 1500-P.P.M. CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID, AS INDICATED BY PERCENTAGE OF GREEN LEAVES REMAINING. CARBOWAX 1500 INCLUDED AT 1.0% CONCENTRATION IN EACH RESPECTIVE CONCENTRATION

GREENHOUSE TEMPERATURE (° F.)	PERCENTAGE OF ORIGINAL GREEN LEAVES REMAINING AT INDICATED NUMBER OF DAYS AFTER TREATMENT															Controls (44 days)
	4 days			14 days			24 days			34 days			44 days			
	500*	1000	1500	500	1000	1500	500	1000	1500	500	1000	1500	500	1000	1500	
75°-90°.....	86	87	87	25	23	23	3	3	0†	0	0	0	0	0	0	62
65°-75°.....	84	84	77	26	23	24	6	6	6	0	0	0	0	0	0	70
50°-65°.....	100	100	100	50	50	48	36	33	32	15	22	20	0	0	0	86
32°-40°.....	97	97	96	80	77	81	59	54	50	47	53	43	45	43	40	69

* Concentration (p.p.m.) of acid in spray.

† 0 indicates entire plant dead.

lished and initiated new vegetative growth. At the end of this period, uniform plants that had developed leafy rosettes 4–6 inches in diameter were grouped into four lots of ninety-six plants for each species. One group of each species was then grown under each of the following temperature ranges: 32°–40°, 50°–60°, 65°–75°, and 75°–90°, respectively. The plants were held at each temperature level for 1 week prior to treatment.

All yellowed or otherwise discolored foliage was removed from the plants at

was a more reliable measure of effectiveness of treatment than was leaf color change.

BARBAREA VERNA

No significant difference in the rate of killing was observed at the two higher temperature ranges (65°–75° and 75°–90°), as shown in table 1. At both ranges the plants responded at about the same rate, and all were dead 34 days following treatment.

At a lower temperature (50°–65°) the plants remained alive significantly long-

er, and an interval of 44 days was required before all the treated plants were dead. None of the treated plants grown at 32°-40° were dead 44 days after treatment (figs. 1, 2), although they lost a higher percentage of leaves than did the controls during this period (table 1). At the end of 44 days, however, the untreated plants grown at 32°-40° had lost 31% of their old leaves and had produced little new growth, while at the next two higher temperature ranges the controls had grown rapidly, had become severely pot-bound, and had started to yellow because of decreased soil fertility.

No significant difference in effectiveness was found between spray applications of 500-, 1000-, or 1500-p.p.m. concentrations of the acid at any of the temperatures used (table 1).

In a comparable experiment, twelve plants of *B. verna* that had been treated with 1000-p.p.m. concentration of the acid and grown for a period of 35 days in the greenhouse at 32°-40° were removed to a warmer house (65°-75°), while twelve others were left at the lower temperature. At the time of shifting, the treated plants had developed some dead leaves (26%) and had stopped vegetative growth but were for the most part green in appearance. Following the change to the higher temperature, all twelve treated plants removed were dead 15 days later (fig. 1), while the treated plants that remained continuously at 32°-40° had changed but slightly in appearance 20 days later. Apparently the acid was present in lethal amounts within or on the surface of the treated plants, but under low temperature (32°-40°) conditions the responses associated with killing were not fully expressed, with the result that the plants remained alive over an extended period at the low temperature but died when subjected to

a temperature ordinarily more favorable for growth.

PLANTAGO LANCEOLATA

An experiment was conducted in the greenhouse with *P. lanceolata* similar to that described for *Barbarea*. Plants treated with 2,4-dichlorophenoxyacetic acid at 500-, 1000-, and 1500-p.p.m. concentration and grown at 65°-75° were killed more quickly than comparable plants treated and grown at 75°-90°. The

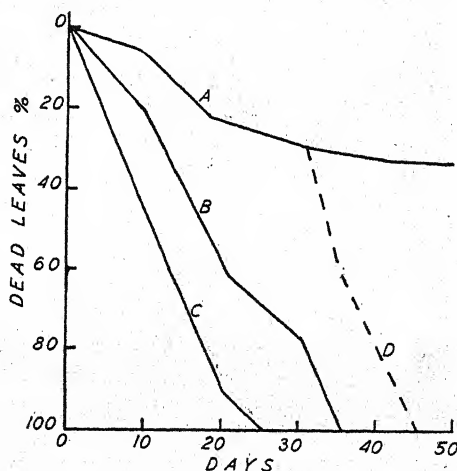


FIG. 1.—Relative rate of killing of *Barbarea verna* growing at different temperatures in the greenhouse following aqueous spray treatment with solution containing 1000-p.p.m. concentration of 2,4-dichlorophenoxyacetic acid and 1.0% Carbowax 1500: A, at 32°-40°; B, at 40°-50°; C, at 65°-75°; and D, at 32°-40° (then after 30 days the plants of this lot were moved to 65°-75° F.).

twenty-four plants included in each treatment at the former temperature were all dead at the end of 23 days, whereas those growing at the latter temperature required 31 days for complete eradication. The control plants grew more rapidly at the lower temperature than at the higher. As was the case with *Barbarea*, no significant difference in rate of killing was observed between the three concentrations of the acid that were applied at the various temperatures.

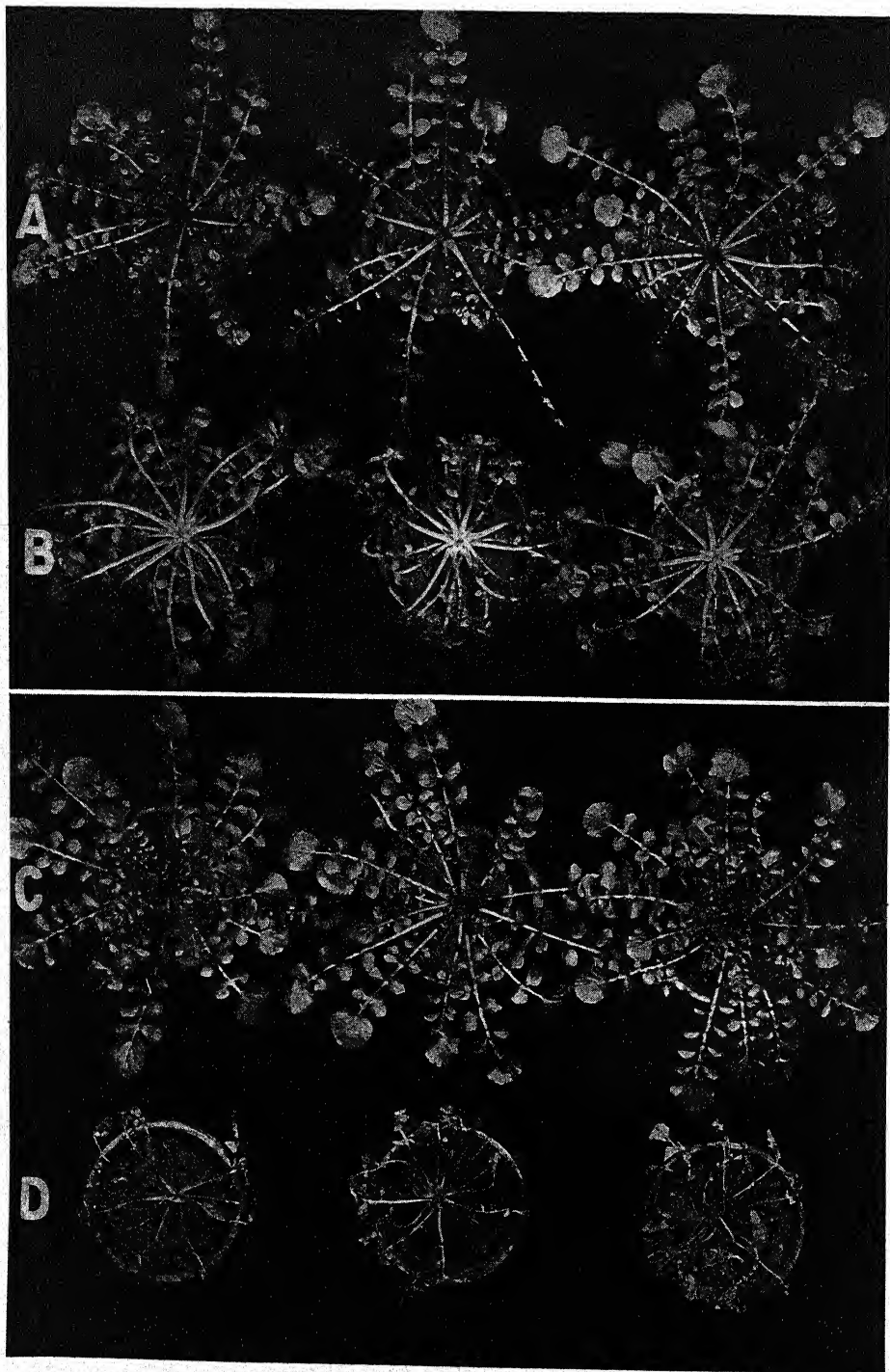


FIG. 2—Effect of aqueous spray containing 1000-p.p.m. concentration 2,4-dichlorophenoxyacetic acid and 1.0% Carbowax 1500 on killing of *Barbarea verna* treated and grown at relatively high and low temperatures in the greenhouse: A, unsprayed, 32°-40°; B, sprayed, 32°-40°; C, unsprayed, 65°-75°; and D, sprayed, 65°-75°. Sprays applied at rate of 5 gallons per 1000 sq. ft. on December 18; photographed 30 days later.

At the end of 21 days the treated plants growing in the greenhouse at 50°-65° showed much more epinasty and rolling of the leaf petioles than those growing at 32°-40°. However, the foliage on all plants at both temperatures was still green at this time. Twelve plants from each treatment were therefore moved from the two relatively low temperatures into a greenhouse at 65°-75°. Following this change, the treated plants previously growing at 50°-65° were all dead within a period of 9 days, while those moved from the 32°-40° range were dead after 14 days. These results further substantiate those obtained with *Barbarea*, that response to the acid is markedly reduced by low temperatures unfavorable for vegetative growth; furthermore, if the growth substance is in or on the plants its effects will be manifested when the plants are subjected to a temperature at which vegetative growth would be resumed.

A field experiment was undertaken on October 7 using *P. lanceolata* growing in Kentucky bluegrass sod. At that time several frosts and prevalent relatively low temperatures had checked the vegetative growth of both grass and plantain. The lawn area used was 70 feet square and contained a dense stand of plantain. It was equally divided into four smaller squares. An aqueous spray solution containing 1000 p.p.m. of the acid and 0.5% Carbowax 1500 was applied to two of the plots with a large power-sprayer at 400-lb. pressure at the rate of 5 gallons per 1000 sq. ft. The remaining two plots were untreated. Observations were made throughout the winter months, and on March 8, 1945, detailed counts of the living plantains found in ten randomized square-yard areas within each plot were obtained.

Although there was slight epinasty

during October, many of the treated plants retained green center leaves throughout November and December. As the temperature slowly began to rise late in February, however, distinct differences between the treated and untreated plants of *Plantago* became apparent. After a few warm days in early March many of the plants were dead. On March 8, 152 days after treatment, the average (ten randomized square-yard areas) numbers of living plantains per square yard in the two spray-treated and the two control plots were as follows: treated plots 2.4 and 2.0, and control plots 47.2 and 30.3, or a decrease in population of 94.9 and 93.4%, respectively, as a result of treatment.

Another field experiment was conducted on *P. lanceolata*, using a plot of turf 128 sq. ft. that contained a dense and relatively uniform stand of plantain. On December 19 it was sprayed, using a knapsack type of hand-pressure sprayer, with a solution containing 1000 p.p.m. of the acid and 0.5% Carbowax 1500 (5 gallons per 1000 sq. ft.). At the time of application the ground was frozen to a depth of 2 inches and the plants were dormant. The leaves of the preceding season were still attached and remained so throughout the 8 succeeding weeks, although some of them had developed brown areas at their tips from slight frost injury. On January 5, 1945, a comparable plot on adjoining turf was sprayed with an aqueous solution containing 1000 p.p.m. of the sodium salt of the acid at the rate of 5 gallons per 1000 sq. ft. No Carbowax was used in this salt solution, but instead a small quantity of soap was added as a spreader.

At 2-week intervals after the treatments had been applied, twelve treated and twelve untreated plants were lifted, the adhering soil thawed out, and the

plants set in composted soil contained in 4-inch pots and placed in a greenhouse maintained at 65°.

There was no evidence of epinasty or rolling of the petioles of the leaves of the dormant plants in turf that had been treated in December with either the acid or its sodium salt, so long as the plants remained outdoors in the frozen ground. Within a few days after the

grown in the field at moderate temperatures and reported earlier (6). Plants brought into the greenhouse after remaining outside at subfreezing temperature for 6 and 8 weeks following treatment, and being subjected repeatedly to ice and snow during this period, responded in a typical way to the growth-regulating substance coincident with the initiation of growth in the un-



FIG. 3.—Killing of *Plantago lanceolata* by aqueous spray treatment with solution containing 1000-p.p.m. concentration of 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500 applied at rate of 5 gallons per 1000 sq. ft. in the field to plants in frozen ground. Sprays applied December 19 and plants moved to 65° greenhouse 17 days later. Photographed January 23, after 18 days in greenhouse. Upper row, unsprayed; lower row, sprayed.

treated plants were brought into the greenhouse they began to show epinastic responses. The control plants grew vegetatively following transplanting.

As the untreated plants developed new growth, the old leaves of the treated ones gradually showed more epinasty, followed by yellowing and ultimately death. As shown in figure 3, the treated plants had developed growth responses which were typical of others treated and

treated plants brought into the greenhouse at the same time. The number of days required for death was significantly less among the treated plants brought into the greenhouse 6 and 8 weeks after treatment than among those brought in 2 weeks after treatment.

OTHER SPECIES

In another experiment, 1-year old plants of *Plantago major* (broad-leaved

plantain) and of *Sisymbrium officinale* (hedge mustard) were transplanted from the field and grown for 1 week at 65°–75°, after which they were treated and maintained at this temperature. Included in the same experiment, plants of *Brassica juncea* (mustard) and *Convolvulus arvensis* (field bindweed) grown from seed planted in the greenhouse in late November were treated when 3–5 inches

treatment (table 2). Under these conditions there was no significant difference between the salt and the acid with respect to their herbicidal activity. Both compounds when applied at 1000 p.p.m. to actively growing plants in the greenhouse resulted in killing of each of the six species investigated. The plants of the mustard family (*Barbarea*, *Brassica*, and *Sisymbrium*) appeared to be slightly

TABLE 2

RELATIVE EFFECTIVENESS OF 2,4-DICHLOROPHENOXYACETIC ACID AND OF ITS SODIUM SALT APPLIED IN AQUEOUS SPRAYS (ON JANUARY 5, 1945) AT 1000-P.P.M. CONCENTRATION, AS INDICATED BY NUMBER OF DAYS REQUIRED FOR COMPLETE KILLING OF ALL PLANTS OF SIX SPECIES GROWING AT 50°–60° AND 75°–90° F. CARBOWAX 1500 AT CONCENTRATION OF 0.5% INCLUDED IN ACID SPRAYS; SMALL QUANTITY OF SOAP INCLUDED IN SALT SPRAYS NOT CONTAINING CARBOWAX. UNTREATED CONTROLS WERE NOT ADVERSELY AFFECTED BY THE TEMPERATURE CONDITIONS IMPOSED

SPECIES	No. PLANTS PER TREATMENT	DAYS REQUIRED FOR KILLING OF ALL PLANTS			
		2,4-dichlorophenoxyacetic acid at 1000 p.p.m.		Sodium salt of acid at 1000 p.p.m.	
		First 21 days at 50°–60°; remainder at 75°–90°	Continuous-ly at 75°–90°	First 21 days at 50°–60°; remainder at 75°–90°	Continuous-ly at 75°–90°
<i>Barbarea verna</i>	15	28	15	28	18
<i>Brassica juncea</i>	20	23	10	25	15
<i>Convolvulus arvensis</i>	16	14	14
<i>Plantago lanceolata</i>	12	31	21	31	21
<i>Plantago major</i>	10	31	18	28	18
<i>Sisymbrium officinale</i>	2	10	12

in height. For comparative purposes, *P. lanceolata* and *B. verna* were also included in this experiment. Spray treatment was with an aqueous solution of 2,4-dichlorophenoxyacetic acid at 1000 p.p.m. containing 0.5% Carbowax 1500, and with the sodium salt of the acid at 1000-p.p.m. concentration.

Under greenhouse conditions, plants that were treated and grown at 65°–75° were all killed within periods of 10–21 days by either the sodium salt or the acid

more sensitive to the acid and were killed somewhat more quickly (2–5 days) than by the application of comparable amounts of the salt.

Rooted pieces of stems of *Stellaria media* (common chickweed) were planted in late November and of *Hydrocotyle rotundifolia* (lawn pennywort) and of *Prunella vulgaris* (heal-all) in the middle of December in flats (12" × 24"). All flats were placed in a relatively cool greenhouse at 65°, and at the end of 45

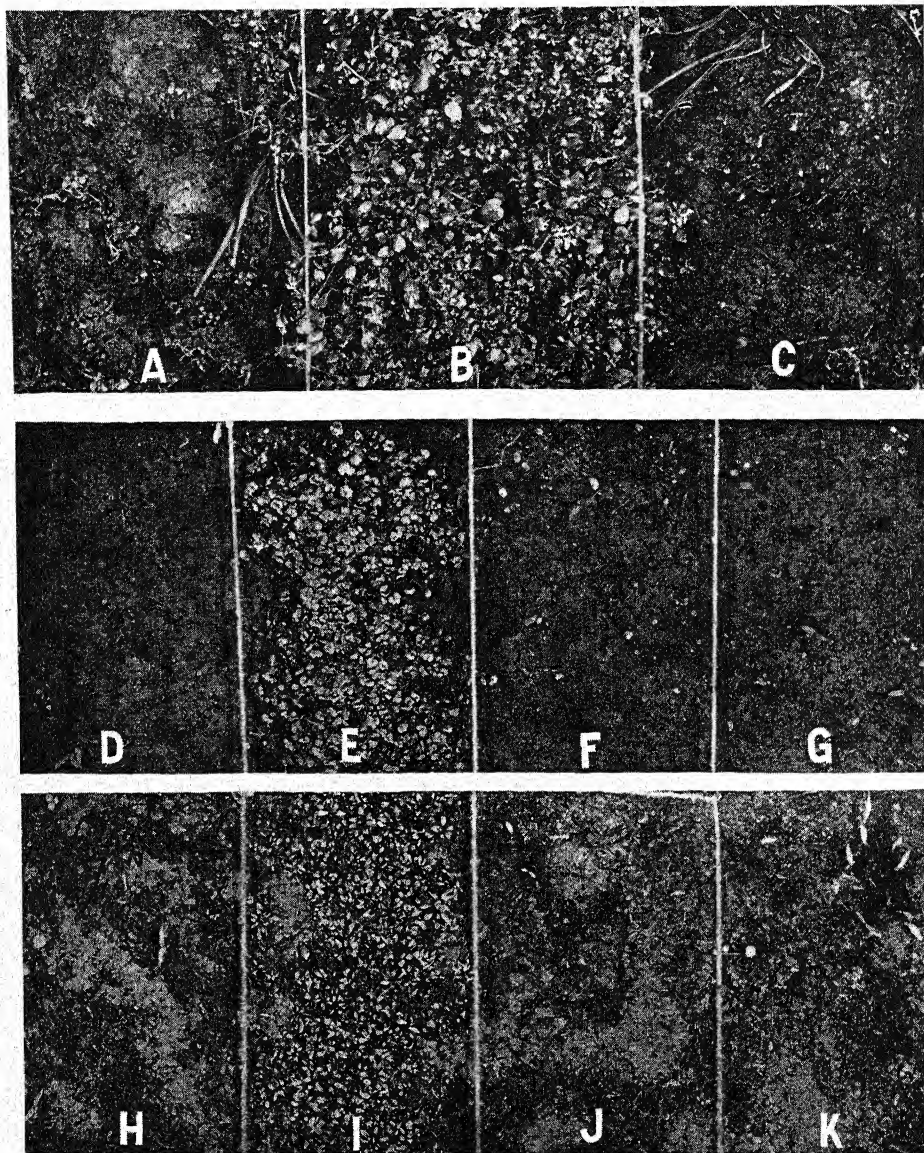


FIG. 4.—Effects of aqueous sprays containing 2,4-dichlorophenoxyacetic acid or its sodium or ammonium salt applied at 1000-p.p.m. concentration at rate of 5 gallons per 1000 sq. ft. to flats in the greenhouse. Upper row, *Stellaria media*; middle row, *Hydrocotyle rotundifolia*; lower row, *Prunella vulgaris*. A, sprayed, sodium salt; B, unsprayed; C, sprayed, acid; D, sprayed, sodium salt; E, unsprayed; F, sprayed, ammonium salt; G, sprayed, acid; H, sprayed, ammonium salt; I, unsprayed; J, sprayed, acid; K, sprayed, sodium salt. Carbowax 1500 at concentration of 0.5% used in the acid sprays; small quantity of soap included in the salt sprays, which were applied without addition of Carbowax.

days the plants were well established. Each of three flats of each species was divided into three or four sections for treatment. For comparative purposes, individual sections in each flat were treated with the acid while others received the sodium or ammonium salt, leaving one section in each flat untreated. A measured volume of aqueous spray was applied in each instance, so that treatment consisted of equivalent amounts of the various compounds at the rate of 5 gallons per 1000 sq. ft. of the spray at 1000-p.p.m. concentration. Carbowax 1500 was included in all of the acid sprays at 0.5% concentration, while the salt sprays were prepared with a small quantity of soap without the addition of the wax spreader.

At the time of treatment the *Stellaria* had made a rank vegetative growth 3-4 inches in height and completely covered the flats, while the plants of *Prunella* and *Hydrocotyle* were growing more slowly and had covered approximately 75-80% of the surface area. After treatment, the flats remained in the 65° greenhouse for a period of 10 days and then were shifted to a warm greenhouse at 75°-80°.

The effectiveness of the ammonium as well as of the sodium salt of the acid was found to be accelerated at increased temperature. This was true also when the compound was applied under the different temperature conditions in the acid form. The *Hydrocotyle* and *Prunella* in a 60° greenhouse failed to show the usual symptoms following treatment with the acid or with its sodium or ammonium salt over a period of 10 days. When the flats were moved to a higher temperature (75°-80°), however, the plants were killed within 4 days (fig. 4). In the same experiment, *Stellaria* that was growing luxuriantly was killed at 65° when treated with either the sodium salt or the acid.

Summary

1. Plants of *Barbarea verna* and *Plantago lanceolata* which were established in 4-inch clay pots in a warm greenhouse (65°-75° F.) were moved to greenhouses in which temperatures of 32°-40°, 50°-65°, 65°-75°, and 75°-90° F. were maintained, and separate lots of twenty-four plants each were spray-treated with 0, 500, 1000, and 1500 p.p.m. concentrations of 2,4-dichlorophenoxyacetic acid 1 week later. Carbowax 1500 was included in the acid sprays.

2. Treatment at the two higher temperature ranges resulted in rapid killing (18-21 days). All three concentrations of the acid were about equal in their effectiveness under the conditions of the experiments. At 50°-60°, however, the time required to kill plants with the acid treatment was extended for 11-15 days longer, while at 32°-40° the treated plants were still living at the end of 50 days following spray applications.

3. The data indicate that the acid was present in lethal amounts, either within the tissues or on the surface of plants, treated in the greenhouse and held at a relatively low temperature (32°-40°).

4. Spray treatments with 1000 p.p.m. concentration of the sodium salt of the acid were about equal to the acid in effectiveness in causing death of *Barbarea verna*, *Brassica juncea*, *Convolvulus arvensis*, *Plantago lanceolata*, *P. major*, and *Sisymbrium officinale*. The salt likewise was found to be present in or on plants treated at low temperature (50°-60°), since upon removal to a warmer temperature they died rather quickly.

5. The ammonium as well as the sodium salt of the acid was effective in killing *Hydrocotyle rotundifolia*, *Prunella vulgaris*, and *Stellaria media*. Death of plants of *Hydrocotyle* and *Prunella* was

hastened by removal from a greenhouse at 60° to one maintained at 75°-80°.

6. Field experiments have demonstrated that dormant *Plantago lanceolata* in frozen ground, having some living leaves, when treated with the acid or its sodium salt (applied in spray solution of

1000 p.p.m. at 5 gallons to 1000 sq. ft.) is killed when the plants are placed under temperature conditions favorable for growth.

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EFFECT OF ALGASTATIC AGENTS ON MARCHANTIA¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 568

PAUL D. VOTH

Introduction

In recent years, certain of the aromatic diamidines have been utilized in the control of protozoan infections and in the control of bacteria in chronic wound sepsis. Propamidine and stilbamidine have received the greater share of attention, and their qualities, especially as they affect the physiology of human beings and of animals, have been described by ASHLEY *et al.* (1), DAUBNEY and HUDSON (4), DEVINE (5), FULTON and

YORKE (6, 7), KIRK and HENRY (8), LOURIE and YORKE (10), THROWER and VALENTINE (12), and by many others. The trypanocidal action of pentamidine has been described by DAUBNEY and HUDSON (4) and by SAUNDERS *et al.* (11). Oxidation of nitrogenous compounds by *Escherichia coli* and other bacteria is inhibited by propamidine (3, 2, 9).

The effectiveness of the diamidines in killing or in retarding the growth of protozoa and bacteria suggested the possibility that these substances might arrest the growth of the common blue-green and green algae which infest ex-

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perimental cultures of *Marchantia* and other plants.

Relatively few species of algae found naturally in the greenhouses at the University of Chicago become a problem in the culture of *Marchantia*. Of the green algae, *Chlamydomonas* sp. becomes established on moist glass cloth within a few days, and *Stichococcus subtilis* (Kütz.) Klerck. is a common contaminant, rapidly developing into yellow-green, stringy masses.

At least two blue-green algae are pioneers in a greenhouse algal succession. *Nostoc muscorum* Born. & Flah. appears as bright blue patches on glass cloth, usually at the bases of recently transplanted tips of *Marchantia*. Another blue-appearing alga is the finely filamentous *Plectonema nostocorum* Gom., which coils into small irregular masses—as seen microscopically. Blue-green algae which appear in great abundance after 3-4 weeks are *Chroococcus rufescens* (Kütz.) Naeg., which is bright blue-green in young cultures but appears brown or black in older ones, and the filamentous, black, *Phormidium autumnale* Gom. The latter grows vigorously and not only surrounds but also covers entire plants of *Marchantia*, actually causing the death of experimental and of stock plants.

When stock cultures are permitted to grow undisturbed for several years with areas of glass cloth unoccupied by *Marchantia*, a climax in algal succession is reached. *Entophysalis cornuana* Sauv. and *Amphithrix janthina* Born. & Flah. constitute most of the $\frac{1}{2}$ -inch algal layer. On soil cultures *Oscillatoria sancta* Gom. is common, but it is not a part of the glass-cloth flora.

The effects of copper salts, potassium permanganate, and the aromatic diamidines—propamide, pentamide, phe-

namidine, and stilbamidine—on the growth of algae and on the vigor of several clones of *Marchantia polymorpha* are reported here.

Material and methods

In preliminary experiments involving the use of copper salts and potassium permanganate, a variety of clones of *M. polymorpha* were tested. In the more extensive trial involving the diamidines, five clones were utilized.

Cultures C and D were male and female plants, respectively, isolated from the charcoal ashes of a camp fire in a second-growth forest near Ohiopyle, Pennsylvania, in June, 1940. These are characterized by a yellow-green color, many gemmae cups, and a dorsally visible midrib. This form of *M. polymorpha* is common from the Atlantic coast to the Black Hills of South Dakota.

Culture Pa (female) originated from plants growing along Stump Spring Road, Big Creek P.O., Huntington Lake, Fresno County, California, at an elevation of 5500 feet. These plants are characterized by a dark-green color, few gemmae cups, a prominent dark-green midrib, and purplish-red lower epidermis and appendages due to the presence of anthocyanin in the cell walls. This clone represents the aquatic form of *M. polymorpha* found locally from Michigan to California and Oregon.

Cultures Q and R (male and female, respectively) originally grew on moss mats and boulders along the water-soaked banks of Deer Creek, Lakeshore, Huntington Lake, Fresno County, California, at an altitude of 7000 feet. These clones grow slowly, usually are yellow-green in color, develop fewer gemmae cups than the eastern form, possess no dorsal midrib line, and are somewhat fleshy. They represent the western form

of *M. polymorpha*. This thallus type has been found in Florida, the Black Hills of South Dakota, Montana, and California. Clones Pa, Q, and R were collected by Dr. Charles H. Quibell in August, 1941.

Through the courtesy of Dr. D. F. Robertson, Merck and Company, four aromatic diamidines were made available. They are:

Propamidine.—4:4'-diamidino-1:3-diphenoxypropane dihydrochloride, $C_{17}H_{20}N_4O_2 \cdot 2HCl$

Pentamidine.—4:4'-diamidino-1:5-diphenoxypentane dihydrochloride, $C_{19}H_{24}N_4O_2 \cdot 2HCl$

Phenamidine.—4:4'-diamidinodiphenyl ether dihydrochloride, $C_{14}H_{14}N_4O \cdot 2HCl$

Stilbamidine.—4:4'-diamidinostilbene diisethionate, $C_{16}H_{16}N_4 \cdot 2C_2H_5O_4S$

Their chemistry has been discussed by ASHLEY *et al.* (1), WIEN (15), and LOURIE and YORKE (10). In the nutrient solution used, all four substances are soluble up to 100 p.p.m., except stilbamidine—which is soluble to about 10 p.p.m.

The culture method described previously (13) consisted of arranging six plant tips on a disk of glass cloth supported by a glass rack and placed in the open half of a large moist chamber. Plants were lightly rinsed with distilled water, after which the appropriate solution was added. Per liter, the stock nutrient solution contained 1.6 ml. KNO_3 , 1.4 ml. $Ca(NO_3)_2$, 1.2 ml. $Mg(NO_3)_2$, 0.8 ml. KH_2PO_4 , and 1.6 ml. $MgSO_4$ —each in 0.5M concentration (14). Micro-nutrients $MnSO_4$, $ZnCl_2$, and $Na_2B_4O_7$ were present in 0.2-p.p.m. and $FeSO_4$ in 0.02-p.p.m. concentrations.

After 12 days of growth on plain nutrient solution, certain cultures were supplied with 100, 10, or 1 p.p.m. of each of these compounds. Control cultures were also maintained. Owing to the severity of the 100-p.p.m. treatment, all such cultures were harvested 22–23 days after planting. Plants on lesser concentrations were grown 28–30 days. For the principal experiment here reported, no. 19,

the natural photoperiod of October prevailed.

When one of the six plants in a culture was preserved in formalin-acetic acid-alcohol, fresh weights of the six plants—and also of the remaining five—were recorded, as well as gemmae-cup numbers and the area (determined photometrically). When one plant was pre-

served, the dry weight of six plants was calculated on the basis of the percentage dry weight of five plants and the fresh weight of six.

Longitudinal and cross-sections were cut at 7–10 μ from sample plants imbedded in paraffin and stained with safranin, gentian violet, and orange G.

Investigation

METALLIC DERIVATIVES

COPPER SALTS.—In February and March, 1943, cupric acetate ($Cu[C_2H_3O_2]_2 \cdot H_2O$) or cupric tartrate ($CuC_4H_4O_6$) was added to the nutrient solution supplied to alga-infested cultures of *Marchantia*. Concentrations of 20, 2, 1, 0.8, and 0.4 p.p.m. were used. Each day the solutions were discarded, the plants and glass cloth rinsed, and new solution containing the specific copper salt added.

In the cultures receiving 20 p.p.m. of either copper salt, all plants of *Marchantia* became dark green with tan borders, and they died after 4 days. When exposed to direct sunlight for a day, the liverworts bleached to a light tan. All algae also bleached, indicating that they too had died. Treatments with 2 p.p.m. reduced the algal population but apparently did not injure the *Marchantia*.

Lower concentrations produced no observable effects. After a week, treatments containing 2 p.p.m. proved to be injurious, but the 1-p.p.m. treatments seemed to loosen the algae from the glass cloth without injury to the liverwort. Eight daily treatments at 1 p.p.m. sufficed to kill all algae in several cultures. Concentrations of 0.8 and 0.4 p.p.m. appeared to inhibit algal growth for a few days, but after a week the glass cloths again became bright green with a fresh algae cover.

In the case of copper acetate, further daily changes of nutrient solution containing 1 p.p.m. resulted in renewed growth of both *Marchantia* and algae. The same concentration of copper tartrate acted differentially, however, in that it suppressed algae and permitted development of the liverwort.

On the basis of these results, most stock cultures were subsequently supplied with 1 p.p.m. of copper tartrate, but during the warm sunny days of early spring the basal portions of most plants of *Marchantia* became tanned, indicating toxic effects of the copper salt. Despite careful rinsing with distilled water and renewal of the nutrient solution without copper salts, all the plants in many cultures died. It is not definitely known whether in *Marchantia* the copper salts are excessively toxic or whether their action is cumulative.

In recent months the effectiveness of cupric sulphate (CuSO_4) as a suppressor of algal growth was determined by employing nutrient solutions containing 1590, 159, and 16 p.p.m. of this salt. All plants of *Marchantia* died within 3 days when the greatest concentration was applied; with 159 p.p.m., necrotic zones appeared on the margins. Both treatments effectively killed all algae. After 21 days of treatment with 16 p.p.m., no severe

injury was evident in the liverwort but all thalli were roughly one-third smaller than in the control plants. Since some algae still persisted at this concentration of copper sulphate, further tests employing copper salts were abandoned.

The watery appearance of *Marchantia* when treated with copper salts at concentrations as low as 1 p.p.m. and the tendency of such salts to kill whole cultures of the liverwort resulted in abandoning the treatments with copper salts, and tests with other substances were initiated.

POTASSIUM PERMANGANATE.—Saturated solutions of this salt were prepared in 500-ml. lots in the moist-chamber halves serving as culture dishes. The entire culture of *Marchantia* attached to the glass cloth was submerged in the saturated permanganate solution for about 5 minutes. The cultures were then removed from the solution, and, after the tips of the plants had been sprayed lightly with distilled water, the excess solution—still colored—was drained off. Washing was continued with tap or distilled water until the drainage water was colorless. Many of the algae adhering to the glass cloth had become a rich brown in color and could be sprayed off rather readily with a stream of water. Older parts of the *Marchantia* thallus became brown or even black, but the growing cells remained alive within a radius of 2–3 mm. of the apical notch—even when treatment had been exceptionally rigorous. For a few days following treatment, especially when high greenhouse temperatures prevailed, growth was negligible. After this initial period of retarded growth, the plants branched and grew rapidly. In stock cultures, where as many as 100 plants are well attached to the glass cloth, the entire cloth surface (except the margins) is soon covered with

the liverwort, thus retarding and preventing the re-establishment of algae.

Although the potassium-permanganate treatment is severe and time-consuming, it is useful in producing robust, algae-free stock plants for experimental use. It would, however, greatly complicate possible interpretation of results of experiments dealing with the effects of variation in nutrient supply on growth.

MANGANESE SULPHATE.—A few clones of *Marchantia* were treated with 1600, 160, and 17 p.p.m. of manganous sulphate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$). The highest concentration killed the liverwort within a week; 160 p.p.m. resulted in much-stunted thalli; and the lowest concentration effected only slight reduction in the width and length of the plants. Algal growth was completely suppressed by the higher concentrations of this sulphate. The 16-p.p.m. concentration effectively reduced the total area of *Marchantia*, but many algae were able to establish themselves on the glass cloth. Since manganous sulphate was ineffective as an algastatic agent, its use was discontinued.

AROMATIC DIAMIDINES

Each of 100 culture dishes, equipped with glass racks and glass cloths, was planted with six tips of *Marchantia* from the appropriate clone. A transplant was 1.1 sq. cm. in area, in the form of an acute triangle with a base of 1 cm. cutting the thallus transversely about 1 mm. in front of the apical notch. The plant fragments were cut with razor blades held in a metal instrument, thus attaining uniformity in size of apical transplant pieces and increasing the speed of planting. All growing points except one were removed from each transplant to insure a still greater degree of uniformity. A few dishes contained an extra plant,

which was preserved in F.A.A. killing fluid at harvest time and subsequently used for anatomical study. All cultures were supplied with the stock nutrient solution for 12 days.

Ten-milligram lots of each of the diamidines—propamidine, pentamidine, phenamidine, and stilbamidine—were weighed out daily and dissolved in 1 liter of nutrient solution. This constituted the 100-p.p.m. solution for each substance. One hundred milliliters of this concentrated solution was diluted with 900 ml. of nutrient to yield a solution with a concentration of 10 p.p.m. Using 100 ml. of the latter solution, another dilution was prepared containing 1 p.p.m. of the respective diamidine. Beginning with the thirteenth day after planting, each culture of six *Marchantia* plants was rinsed with a spray of distilled water, the old nutrient solution discarded, and about 115 cc. of the proper solution added daily. All dishes were compactly arranged on a large table and rotated in a regular manner every third day to minimize differences in light intensities and in evaporation rates in marginal and central dishes.

After 1 week, plants receiving 100 p.p.m. pentamidine began to show marginal necrosis. Clone Q was affected earliest and most severely. At harvest time, plants of this clone were roughly ovate in shape, with much-constricted terminal growing lobes. The broadest portion had developed before treatment with pentamidine began. Hence in Q, approximately only 15% of the thallus area was formed in the 11 days of pentamidine treatment. The gross appearance of all clones after treatment with the diamidines is summarized in table 1.

As judged by the color of the glass cloths, only two substances—propamidine and pentamidine—suppressed all

algal growth when applied in concentrations of 100 p.p.m. In treatments with phenamidine or stilbamidine, in the same concentration nearly every glass cloth became at least slightly green, indicating the presence of green algae and possibly of *Chroococcus*. As already noted, pentamidine not only suppresses algal growth but also causes the margins of *Marchantia* thalli to die and turn tan in color. Thus propamidine, which is able to reduce algal growth to a minimum and

and dry weight are greater. While this characteristic seems to be desirable, it will be shown subsequently that lower concentrations of this material are less effective in controlling the growth of algae than are propamidine and pentamidine.

Gemmae cups tend to appear in greater numbers on male (C, Q) than on female plants (D, R), as reported for other clones in previous experiments (13, 14). Clone Pa, which originally grew in a semi-aquatic habitat, rarely produces

TABLE 1
GROSS APPEARANCE OF MARCHANTIA CLONES GROWN ON 100 P.P.M.
OF THE SUBSTANCES FOR 11 DAYS

Treatment	Approximate area alive or green (%)	Algae	Type of necrosis	Additional observations
Propamidine	25	None	Basal and general	Color of dry oak leaves
Pentamidine	75 in C, 25 in others	None	Marginal in Pa, Q, R	Dark tan color
Phenamidine	10 in D, 75 in others	Few	Small spots (nearly total in D)	Black tips or black spots near notch in C, Pa, Q
Stilbamidine	95	Few	None except in D	Color bright green (D pale green)
Control	100	Usually many	No necrosis	Tips of some Pa plants watery

yet not cause local or general necrosis of *Marchantia*, gives the greatest promise of being a desirable algastatic compound.

Effects of the four diamidines on area and dry weight of *Marchantia* are presented in table 2.

Plants in cultures having a concentration of 100 p.p.m. of propamidine, pentamidine, or phenamidine attained about one-half (or in some instances about one-fourth) the size of control plants. As is to be expected, the dry weight of these plants is also much less than that of the control plants—as little as one-half or only one-third. In contrast to the other diamidines, with stilbamidine the area

gemmae cups, even when the proportions of anions and cations are varied.

Marchantia plants growing on 10- and 1-p.p.m. concentrations of the diamidines were permitted to grow an additional week before being harvested, so that each culture received twenty doses of the respective diamidine. Dry weights of clones C, D, Q, and R are plotted in figures 1-4. Two cultures of six plants each of the four clones were grown on plain nutrient solution as the respective controls for all four treatments.

The effects of propamidine, pentamidine, and phenamidine on the growth of *Marchantia* are strikingly similar (figs.

TABLE 2

EFFECT OF ADDING 100 P.P.M. OF A DIAMIDINE TO NUTRIENT SOLUTION SUPPLIED TO SIX PLANTS OF *MARCHANTIA POLYMORPHA*. FOR CLONES C, PA, AND Q, TWO LOTS OF SIX PLANTS EACH WERE AVERAGED

Treatment	Area (sq. cm.)	Dry weight (cgm.)	No. of gemmae cups
Clone C♂			
Propamidine.....	21.0	8.0*	13
Pentamidine.....	26.5	8.4*	10.5
Phenamidine.....	22.5	11.4*	14.5
Stilbamidine.....	35.0	14.4*	24
Control.....	55.5	20.5*	35
Clone D♀			
Propamidine.....	26.0	7.3	14
Pentamidine.....	27.0	8.6	7
Phenamidine.....	22.0	8.7	9
Stilbamidine.....	28.0	10.8	12
Control.....	77.0	22.0*	45.5
Clone Pa♀			
Propamidine.....	27.0	8.5	1
Pentamidine.....	28.0	9.6	0
Phenamidine.....	30.5	12.8*	0
Stilbamidine.....	34.5	13.2	0
Control.....	80.0	19.2	0
Clone Q♂			
Propamidine.....	23.0	9.2*	7.5
Pentamidine.....	24.5	10.2*	6
Phenamidine.....	21.5	12.0*	10
Stilbamidine.....	33.0	14.4*	22.5
Control.....	41.0	17.4*	21.5
Clone R♀			
Propamidine.....	23.0	9.9	3
Pentamidine.....	25.0	9.9	2
Phenamidine.....	30.0	13.6	4
Stilbamidine.....	31.0	14.3	10
Control.....	44.0	16.7	11

* Calculated dry weight (at least in part) based on five plants.

1-3). With the exception of the dry weight of clone R grown on 10 p.p.m. propamidine, clones C, D, and R show a relatively low weight on 10 p.p.m., a high weight on 1 p.p.m., and an intermediate weight on the control.

At the time of harvest, the distribution and relative abundance of the algae in cultures treated with the lower concentrations of the diamidines and in the control were noted. Some algae appeared in all cultures. Only a faint green color was apparent at the margins of glass cloths in cultures treated with 10 p.p.m. propamidine and 10 p.p.m. pentamidine. In the latter treatment the basal margins of the plants were dry, again indicating the tendency of this compound to kill peripheral areas of the liverwort. Glass cloths in cultures receiving 10 p.p.m. phenamidine were uniformly covered with a conspicuous layer of green and blue-green algae. One culture of D treated with 10 p.p.m. stilbamidine developed no algae, but all others similarly treated supported an abundant algal cover.

Cultures treated with 1 p.p.m. of the four diamidines all supported some algae. Propamidine, pentamidine, and phenamidine cultures were about equally effective in controlling growth of the green algae. Cultures Q and R treated with either of these three substances were rather heavily infested with blue-green algae, especially *Phormidium* and *Chroococcus*. Nevertheless, the dry weight of R in each instance was greater than the weight of this clone when grown on the control solution. Cultures of C and D treated with phenamidine and stilbamidine in 1-p.p.m. concentration also were infested with *Phormidium* and other blue-green algae. The algastatic action of these compounds

in this minute concentration permitted a marked increase in dry weight, suggesting that the optimum benefit to be derived from these substances is near 1 p.p.m.

Control cultures were all heavily infested with all algae encountered in this investigation.

Clone Q appears to be a special problem, since it reacts unfavorably to all four diamidines with striking uniformity. Additional tests are desirable to determine whether this behavior is character-

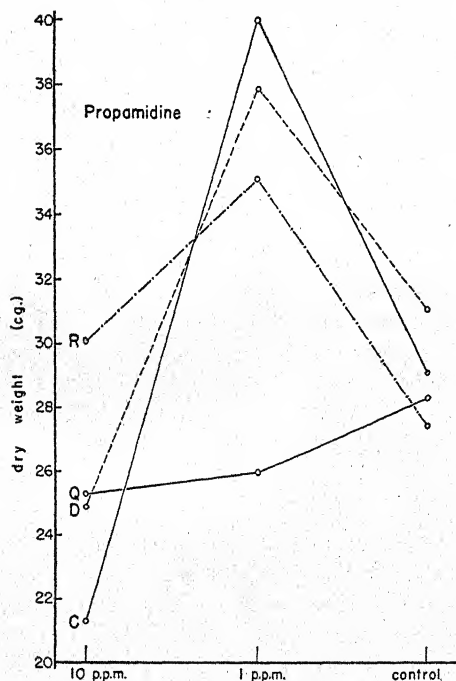


FIG. 1.—Dry weights of four clones of *Marchantia* grown on 10 and 1 p.p.m. propamidine and on untreated nutrient solution. Weights of clones D and Q (10 p.p.m.), C and Q (1 p.p.m.), and all control plants are based on two cultures of six plants each; other weights by one culture.

istic at all seasons of the year. It is possible that *Phormidium autumnale* and similar algae were growing with the central strand of pegged rhizoids and thus

were in a favorable position to contaminate all cultures with a vigorous mass of trichomes. Plants of this clone growing in one dish and treated with 1 p.p.m. stilbamidine developed an almost pure

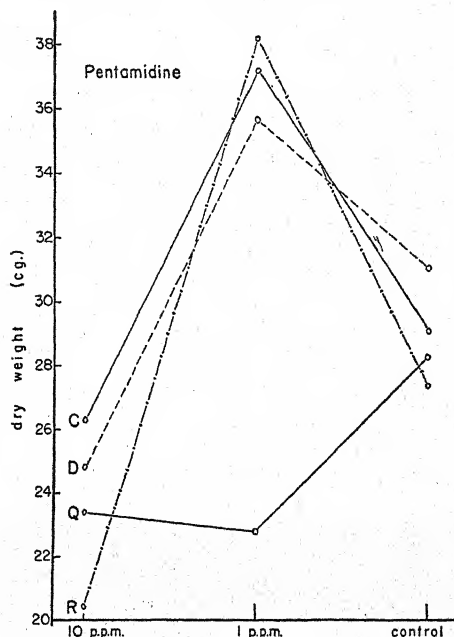


FIG. 2.—Dry weights of plants of *Marchantia* grown on dilute solution of pentamidine and on untreated nutrient solution. Weights of clones D and R (10 p.p.m.), C and Q (1 p.p.m.), and all control plants are based on two cultures of six plants each; others on one culture.

culture of a diatom, *Nitzschia communis* var. *obtusula* Grun., which covered the glass cloth completely except for two small raised areas. Dr. Ruth Patrick kindly identified this plant.

Stilbamidine reacted somewhat differently from the other diamidines. In 100 p.p.m. concentration it depressed the growth of *Marchantia* less than the other compounds. In concentrations of 10 and 1 p.p.m. the effects of this substance are somewhat variable, as is shown in figure 4. As plotted, in all three treatments

clone C was infested with *Phormidium* and other algae, thus accounting for its low dry weights. In contrast, the cultures of clone D had developed only a few algae, mainly green, permitting an unusually vigorous development of its plants. Clones Q and R followed their respective patterns of response, as judged by treatments with other diamidines. On the basis of the results recorded in tables

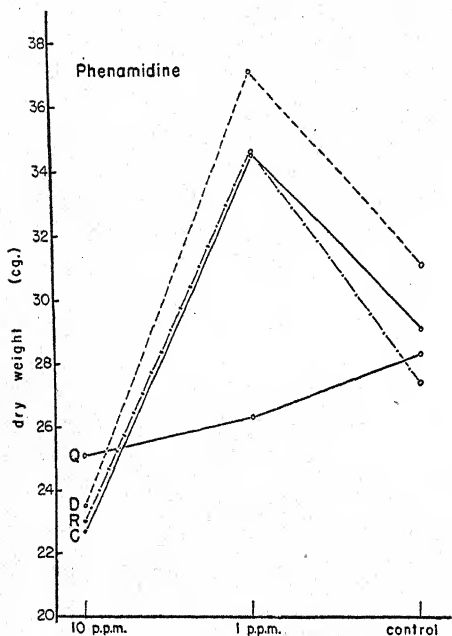


FIG. 3.—Dry weights of plants of *Marchantia* grown on dilute solutions of phenamidine and on untreated nutrient solution. Weights on same basis as in fig. 2.

1 and 2 and in figure 4, stilbamidine is not an effective algastatic compound.

To test the ability of the diamidines to kill algae or to suppress their growth, two series of cotton-plugged 15-cm. test tubes were prepared. Some tubes contained 10 ml. of control nutrient solution; others contained 1-, 0.1-, or 0.01-p.p.m. concentrations of each of the four diamidines. Each test tube of one series was inoculated with several drops of a

dark-green suspension consisting mainly of *Chlamydomonas*. In the second series no inoculations were made; instead, nutrient solution which had been exposed to greenhouse conditions for several hours was used in making up the various concentrations of diamidines. After one month (November, 1944), the relative turbidity of the solutions was noted. In the inoculated series of test tubes no conspicuous or consistent differences in turbidity and green color were discernible. Control cultures, as well as those containing 10–0.01 p.p.m. of one of the four diamidines, ranged from 50 to 100% in turbidity, using the greatest density as a standard.

In the series prepared without inoculation, four control tubes and those containing 0.01 p.p.m. propamidine, 0.01 p.p.m. pentamidine, 0.1 and 0.01 p.p.m. phenamidine, or 0.1 and 0.01 p.p.m. stilbamidine were 50% turbid. Tubes containing concentrations of 10 and 1 p.p.m. of propamidine and pentamidine and 10 p.p.m. of phenamidine and stilbamidine were entirely devoid of green color. The remaining tubes, containing lesser amounts of the diamidines, were slightly turbid. From these trials on *Chlamydomonas*, and from extensive use of the diamidines in the culture of stock plants of *Marchantia*, it is obvious that none of these substances is able to eliminate a copious growth of algae from a culture at concentrations which are not also detrimental to the liverwort. When algae are few in number, a culture of *Marchantia* may be kept relatively free by applications of 1 p.p.m. of propamidine or pentamidine.

To determine the possible effect of propamidine on initiation and growth of rhizoids, gemmae of several clones representing the eastern, western, and aquatic forms of *Marchantia* were planted in agar

plates. Rhizoids formed as readily and as extensively in treatments at 100, 10, and 1 p.p.m. as in control plates. After about a week, gemmalings receiving 100 p.p.m. propamidine were stunted, their margins upturned, and their color a dull, translucent green. Propamidine does not therefore affect the development of rhizoids but instead affects the aerial portions, especially the margins of the thallus, where photosynthetic tissue is predominant.

Of special interest is the absence of tumors, regenerated areas, and other malformations in the thallus of *Marchantia* when treated with diamidines in concentrations of 100 p.p.m. or less. Approximately 150 microscopic slides were prepared of longitudinal and cross-sections of the forms of *Marchantia* used under every experimental condition here reported. The drying of the older parts of the thallus and the margins in treatments with higher concentrations of pentamidine seems to be no different histologically from the drying of the exposed margins of the control plants. No conspicuous reduction in cell size was discernible, suggesting that the narrower thalli occurring in the 100-p.p.m. treatments may be due to fewer cell divisions in the meristematic region of the apical notch. The shorter thalli probably result from fewer divisions of the apical cell.

Discussion

Additional tests are desirable to determine the specific relationships between low concentrations of the diamidines and the ability of plants similar to *Marchantia* to utilize fully anions (such as nitrates, phosphates, and sulphates), and also the cations, commonly supplied to plants. WIEN (15) reported that additional calcium decreases the fall in blood-pressure in animals treated with the

diamidines here discussed. No information of any direct physiological effects on the cytoplasm in *Marchantia* is available. The constriction of the thallus of *Marchantia* treated with 100 p.p.m. of pentamidine resembles the narrowing of plants grown on solutions high in calcium

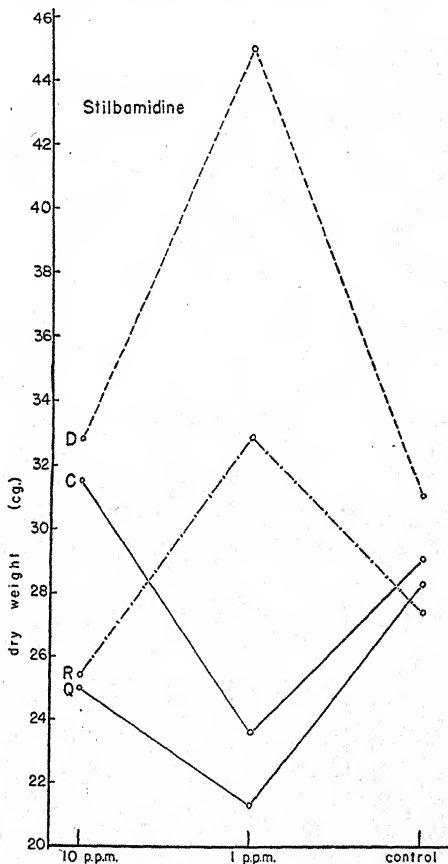


FIG. 4.—Dry weights of plants of *Marchantia* grown on dilute solutions of stilbamidine and on untreated nutrient solution. Weights of clones D and R (10 p.p.m.), C and R (1 p.p.m.), and all control plants are based on two cultures of six plants each; others on one culture.

and lacking in potassium and magnesium (13).

Stilbamidine solutions subsequent to exposure to light for even a few hours are known to be toxic to man and animals (7, 8). No such toxic effects were evident

in the present experiments. Even though freshly prepared each day, the solutions were in open dishes and exposed to sunlight under greenhouse conditions. From the results reported here, stilbamidine is less toxic to algae and *Marchantia* than are the other three diamidines.

If in further tests a 1- or 2-p.p.m. concentration of propamidine interferes less with the growth of *Marchantia* than with interfering algae, investigations should be extended to determine the effect of this and other diamidines on the growth of moss protonemata and fern prothallia. An algastatic compound for use in cultures would be of value.

Summary

1. Green and blue-green algae which contaminate greenhouse cultures of *Marchantia* are named.
2. Copper acetate, tartrate, and sulphate are not sufficiently differential in their action, killing *Marchantia* as well as the algae they are to control.
3. Potassium permanganate in a saturated solution is useful in killing algae in stock cultures. Since all older portions of the *Marchantia* thallus are also killed, this treatment is not recommended for the control of algae in experiments involving mineral nutrition.

4. Propamidine, pentamidine, phenamidine, and stilbamidine stunt the growth of *Marchantia* and suppress algal growth at 100 p.p.m. At 10 p.p.m. most clones of *Marchantia* produce as much dry weight as the control plants. Algal growth is slight on treatments with propamidine and pentamidine; extensive on treatments with the other two diamidines. Treatments with 1 p.p.m. result in the production of much more dry weight in *Marchantia*, while many algae appear in small amounts in the cultures.

5. Propamidine interferes least with the growth of *Marchantia* and is as effective an algastatic agent as the other diamidines. Pentamidine produces marginal necrosis on *Marchantia* in the higher concentrations. Stilbamidine is the least effective of the diamidines tested.

6. Clone Q, a western form of *Marchantia*, failed to benefit from treatments with the diamidines.

The assistance of Mr. ROBERT BANDURSKI in many of the experiments is gratefully acknowledged. The algae were identified through the co-operation of Dr. FRANCIS DROUET of the Chicago Natural History Museum.

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CYTOHISTOLOGY OF THE REACTION OF MAIZE SEEDLINGS TO COLCHICINE¹

JOHN E. SASS AND JOHN M. GREEN

Introduction

The production of autotetraploid plants by means of colchicine has become an established technique in plant breeding (1-5, 8). The doubling of the chromosome number is known to be the result of an inhibition of the anaphase mechanism, followed by the incorporation of a polyploid chromosome complement in a restitution nucleus (4). The specific action of colchicine on organ primordia, developing organs, tissues, and tissue systems is in need of further study in diverse taxonomic categories.

Relatively meager success has been achieved in attempts to double the chromosome number of maize with colchicine. The present study was undertaken to determine some cytohistological features of the responses of maize seedlings to colchicine in relation to the erratic production of tetraploidy.

Material and methods

Iowa B1, an inbred line of yellow dent maize, was used for this study. Treatment of the seedlings was started when the third leaf was partially unrolled. The method used has been described by RANDOLPH (7) and consists of immersing the cut end of the radicle in a 0.05% aqueous solution of colchicine. The treatment may be continuous or may be alternated with immersion of the roots in water. The two methods have yielded similar results. The selected plants were subjected to continuous treatment for 91 hours, terminating when a majority of the seedlings were visibly swollen in the region of the coleoptile node (fig. 1). The identification of tetraploid plants in the field was based on pollen size and progeny tests.

Microscopic studies were made by the use of microtome sections of root tips and stem tips. The excised tissues were killed in a chrome-acetic-formalin solution, dehydrated with a dioxan series and imbedded in paraffin. Root-tip sections were stained in gentian violet-iodine or iron-haematoxylin. Stem-tip sections

¹ Contribution from the Iowa Agricultural Experiment Station, Ames, Iowa, in co-operation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U.S. Department of Agriculture. Journal Paper no. J. 1272 of the Iowa Agricultural Experiment Station, Project 182.

were stained in iron-haematoxylin for cytological details and in safranin-fast green for histological features.

Morphological and cytohistological abnormalities

Maize seedlings treated by the foregoing method show three gross morpho-

mately 200 plants were examined in 1943. Thirty-six of these plants yielded abnormally large, apparently $2n$ pollen. The average diameter of a sample of this large pollen was $127 \pm 1.87 \mu$, whereas a sample from a diploid sib had an average diameter of $96 \pm 0.68 \mu$ (fig. 2), corroborating the results obtained by RAN-

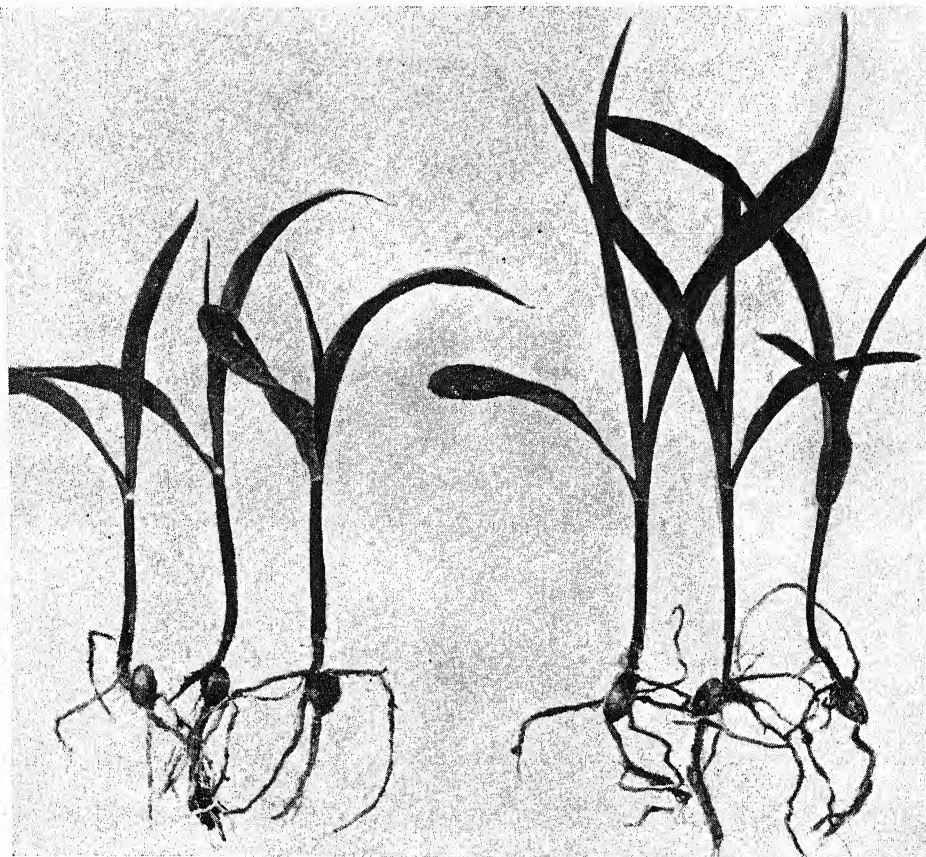


FIG. 1.—Seedlings of maize: plants at left treated with colchicine, normal check plants at right

logical abnormalities: stunting of the entire plant, enlargement of the nodes (fig. 1), and enlargement of the root tips. Treated seedlings have high mortality, and the survivors recover slowly.

A low incidence of tetraploid sectors was found in the floral parts of treated plants. Pollen samples from approxi-

DOLPH (6). Three of the progenies grown from selfed seed of these thirty-six plants consisted of tetraploids. The fact that the remaining progenies were apparently normal diploids is interpreted as evidence of sectoring in the tassel and possibly in the ear. RANDOLPH (6) has reported that the pollen of tetraploid

plants does not compete favorably with the pollen of diploid plants; therefore, in a pollen mixture from a tassel containing $4n$ sectors, pollination would be effected by the monoploid pollen.

A prominent histological feature of the thickened root tips of treated plants is the small size of the root cap (fig. 3). Mitosis and excessive cell expansion occur throughout the promeristem, pro-

some number, which show that the giant nuclei are polyploid. Chromosome counts from metaphase figures afford further evidence of polyploidy. Anaphase figures show much irregularity, particularly multipolar spindles, irregular separation of chromosomes into unequal groups, and excessive clumping of chromosome groups. Telophase reconstruction of the incompletely separated chromosome

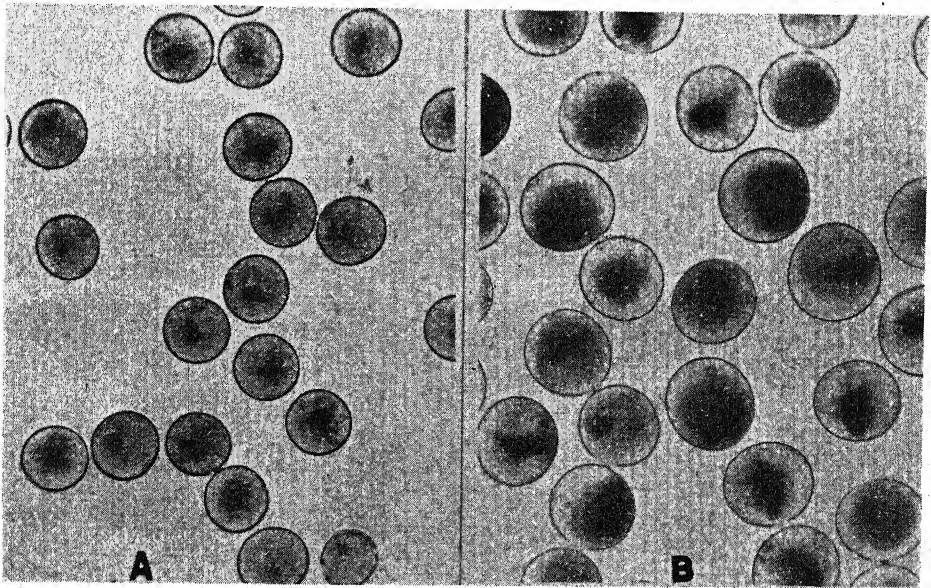


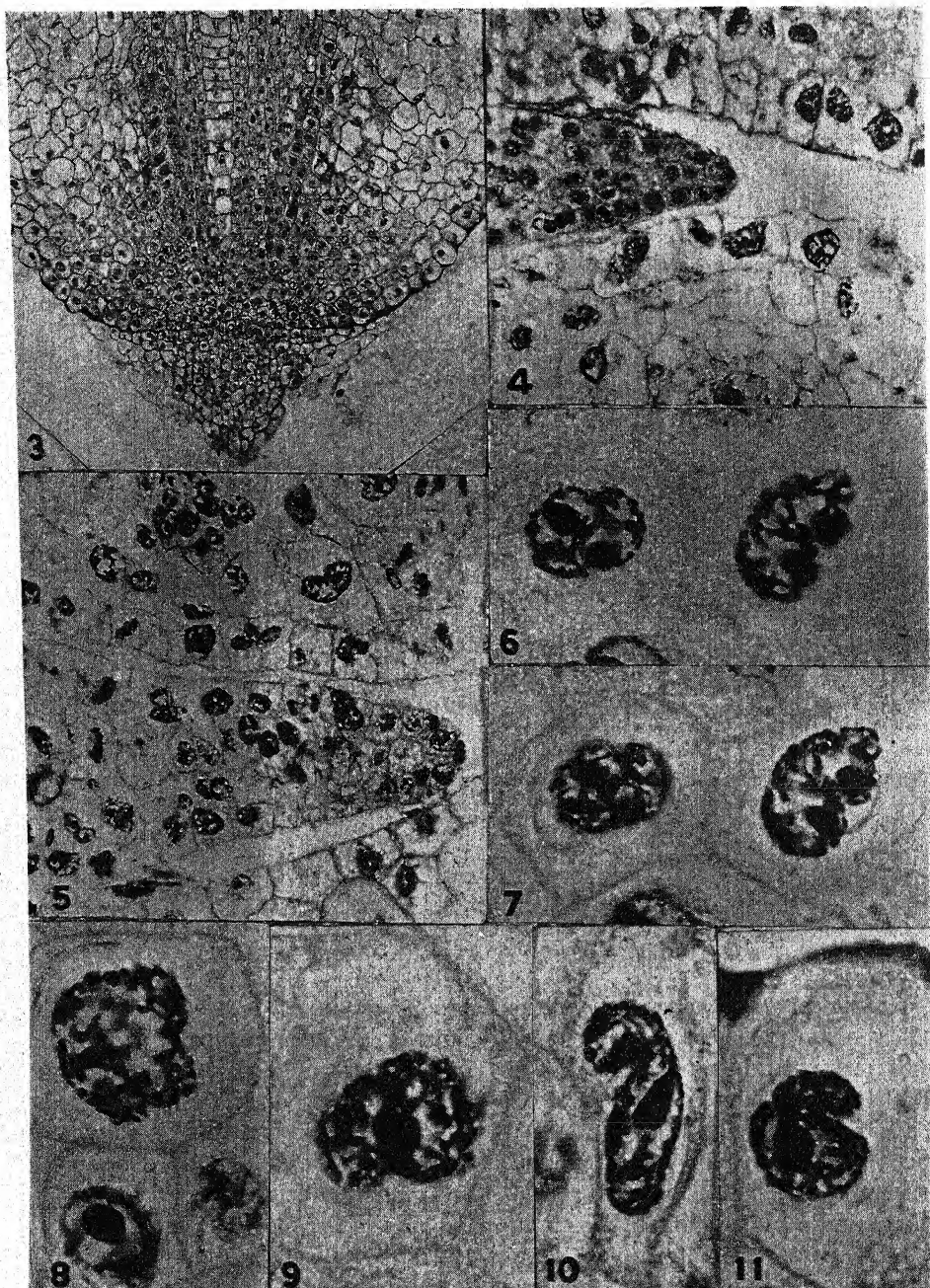
FIG. 2.—*A*, normal monoploid pollen; *B*, large diploid pollen

ducing the greatly thickened condition of the root tips, whereas the root cap remains very small, apparently as the result of inhibited mitosis and limited cell expansion.

Sections of such root tips exhibit numerous abnormal nuclei, which are usually large and variously lobed. All gradations may be observed, from very slight indentation to complete separation into two or more nuclei (figs. 6-11). When these large nuclei are in late prophase (fig. 8), the chromosomes are sufficiently distinct to permit estimates of chromo-

groups results in the formation of the more or less lobed polyploid nuclei (figs. 6-11). Complete separation of chromosome groups produces the multinucleate condition. Abnormal mitotic activity is most evident in a zone of the dermatogen and outer perilem, near the thin sloughing edge of the root cap (fig. 3).

The cytological abnormalities observed in the root tip were also found in the plumule. Sections of the promeristem of the stem of the seedling show relatively few abnormal nuclei, and the stem tip is a smooth dome. On the other hand,



FIGS. 3-11.—Fig. 3, longisection of root tip of treated plant, showing small root cap on swollen root. Fig. 4, transection of apparently normal marginal meristem of a leaf and affected surface cells of adjacent leaf tissues. Fig. 5, thickened marginal meristem and adjacent leaves, both showing nuclear abnormalities. Figs. 6-11, abnormal polyploid nuclei of treated plants.

the small, post-embryonic leaf primordia exhibit a striking response. The surface and subsurface cells become greatly enlarged, producing considerable thickening and a highly spongy cellular texture in the margin of the leaf primordium, especially on the distal edge (fig. 12). These leaf primordia exhibit to a marked degree the nuclear abnormalities previously described.

Aberrant mitosis also occurs in localized regions of the seminal leaves, which, except for the meristematic margins, are relatively more differentiated than the newly formed leaves. Abnormal mitosis occurs in the epidermis (fig. 4) and in the mesophyll (fig. 5). In figure 4 the marginal meristem of a leaf appears to have the normal thin edge, and the cells and nuclei are of normal size, whereas the older tissues of the adjacent leaf show a zone of expanded cells and giant polyploid nuclei. The marginal meristem in figure 5 obviously is abnormally thickened and shows nuclear abnormalities.

This study of the reactions of meristematic organs and histogens has shown some specificity of response. Inhibition of meristematic activity and of cell expansion in the calyptragen contrasts sharply with the continued, though abnormal, mitotic activity in the adjacent promeristem and with the marked lateral cell expansion in the promeristem, plerome, and periblem. The latter condition is in accord with the observations of HAWKES (4), who ascribed transverse expansion and lack of extensive linear elongation of cells to a loss of cell polarity.

The erratic localization of the formation of tetraploid cells, particularly in the edges and thin portions of young leaves, in contrast with the scarcity of tetraploid cells in the massive promeristem, suggests that the tassel primordium

as a whole is unlikely to be affected. It is more probable that the thin floral primordia are affected, resulting in the known production of tetraploid branches of the tassel. Cytohistological verification must await the study of field-grown plants during the period of inflorescence, floret, and floret-organ initiation.

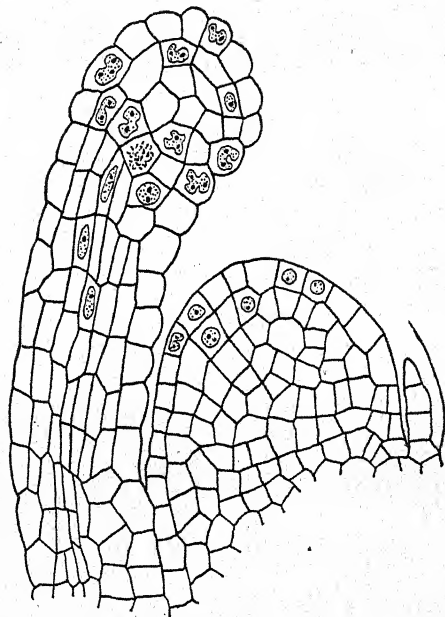


FIG. 12.—Stem tip and post-embryonic leaf of treated plant, showing swollen leaf tip and abnormal nuclei (semidiagrammatic).

Summary

1. Seedlings of maize were treated with colchicine. Morphological abnormalities consisted of stunting of the seedling and swelling of the coleoptile node and of the root tip.

2. Sections of the root tip and plumule exhibit parallel mitotic aberrations—for example, multipolar spindles, clumping of chromosomes, multinucleate cells, and giant polyploid nuclei having varying degrees of lobing. These responses are

especially prominent in the marginal meristems of leaf primordia, the epidermis and mesophyll of young leaves, and in both the plerome and periblem of root tips.

3. The massive promeristem of the

plumule is relatively unresponsive; cell expansion in the calyptrogen appears to be inhibited.

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CURRENT LITERATURE

Problems in Tree Nutrition. By M. C. RAYNER and W. NEILSON-JONES. London: Faber & Faber, Ltd., 1944. Pp. 184. 12/6.

This book presents detailed experiments on the use of various substances to enhance the value for the production of coniferous trees of certain British soils at Wareham Forest, Dorset. The correlation of growth and development of some species of conifers and oaks with the presence or absence of mycorrhiza, and the inoculation of soils with composts containing certain fungi, are discussed.

In the particular soils experimented upon, the presence of substances actively inimical to growth was demonstrated and the origin of the resulting toxicity confirmed. The toxins operate directly by inhibiting fungal growth and by almost complete cessation of cellulose decomposition. The indirect effects of such biological inertia are the restriction of root growth of trees, impeding of mycorrhiza formation, and curtailment of supply of nutritive requirements.

Justification for the use of organic composts for relieving infertility under field conditions has been provided by laboratory experiments proving that removal of toxicity and profound alteration of the organic substrate and soil bionomics follow addition of compost. Changes so induced are self-propagating and the effects on growth persistent.—E. J. KRAUS.

Hayfever Plants. By ROGER P. WODEHOUSE. Waltham, Mass.: Chronica Botanica Co., 1945. Pp. 245. Illustrated. \$4.75.

This volume supplements a previous one by the same author (*Pollen Grains*, McGraw-Hill, 1935) on pollen grains of plants concerned with hayfever. Brief descriptions of the entire plants are given, and their geographical ranges and those of some of their near relatives are briefly noted. Some of the species are illustrated by means of line drawings, many of which are critical and helpful toward identification, others are too sketchy to be of any diagnostic value. A chapter on regional surveys, designed to furnish information on the hayfever plants occurring in various regions of the United States and on the period of the year during which the various species are of significance, presents available information in a condensed and usable form.—E. J. KRAUS.

Weeds of Lawn and Garden. By JOHN M. FOGG, JR. Philadelphia: University of Pennsylvania Press, 1945. Pp. 215. Illustrated. \$2.50.

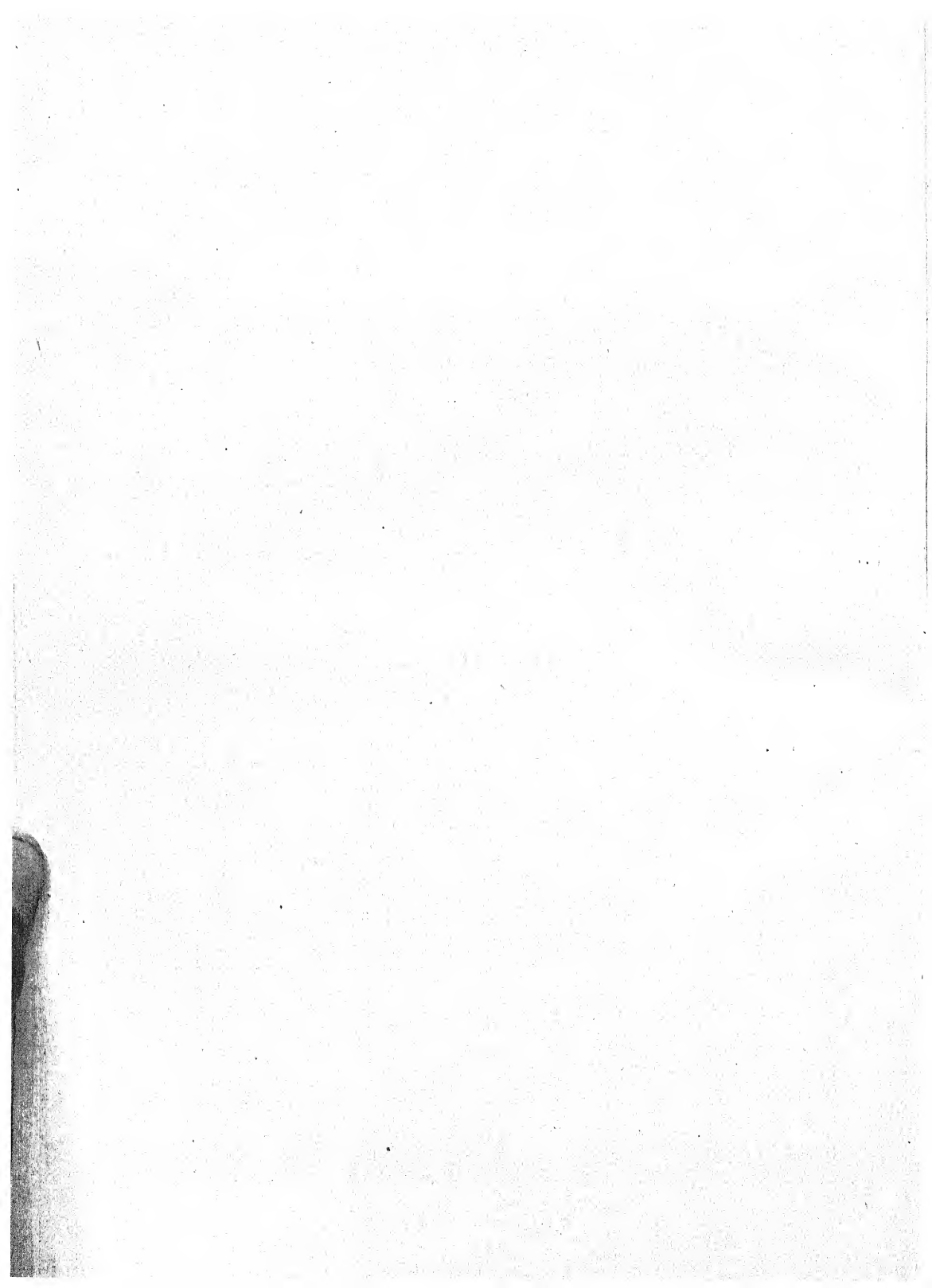
This readable handbook lists more than 175 species of plants which are likely to be encountered as garden and field weeds in eastern temperate North America. In addition to some material of general interest, each species is described in some detail, and the likely places where it may occur are indicated.

The line drawings accompanying the descriptive matter are well done, but it is doubtful whether the hopes of the author that the drawings will be of special aid in the recognition of specific weeds are likely to be realized. The illustrations are much more likely to be of value to the botanist than to the average gardener, whose experience scarcely enables him to recognize species by comparison with line drawings. The descriptive material is concise and pertinent.—E. J. KRAUS.

The Permeability of Living Cells. By S. C. BROOKS and MATILDA MOLDENHAUER BROOKS. Ann Arbor: Edwards Brothers, Inc., 1944. Pp. ix+396. Illustrated. \$5.00.

This book is in a field in which the authors have already made many important contributions. It was published originally as one of the *Protoplasma Monographs*. The literature on the important theories of permeability and the main factors affecting it is summarized and critically evaluated. Seven chapters consider the permeability to water and the water and osmotic relations of plant and animal cells. Among the animal tissues considered are erythrocytes, eggs of marine invertebrates and of vertebrate animals, protozoa, muscle cells and nerves; also, the osmotic relations of bacteria, algae, fungi, and vascular plants are discussed. Five chapters deal with the permeability of living tissues to non-electrolytes, weak electrolytes and gases, strong electrolytes, and dyes, including a chapter on the oxidation-reduction dyes. The accumulation of ions by cells, especially plant cells, is considered at length.

There has long been need of a book of this kind, and biologists will welcome this critical résumé of the literature on permeability. The bibliography contains about 1400 references.—S. V. EATON.



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